1. Introduction

Soon after it was discovered in the mid-sixties that lipids, as the core ingredients of cellular membranes, can form artificial vesicles,[1] many laboratories, notably the laboratory of Erich Sackmann, began to use the vesicle as a possible model to study the physical basis of cellular processes.[2] The use of giant vesicles of sizes comparable to the sizes of cells, and thus observable by optical microscopy, revealed the propensity of vesicles to bud, that is, to attain shapes composed of spherical parts connected by tiny necks or narrow tubes.[3–6] Examples of vesicles with such shapes are shown in Figure 1. Formation of membrane buds also occurs in many cellular processes (Figure 2). The similarities between the two systems are evident, which suggests that studies of this phenomenon in vesicles may contribute to an understanding of the related biological processes.

This Minireview provides an appropriate opportunity to demonstrate the connection between the results of some early experimental and theoretical investigations of vesicle budding and the more recent application of the concepts developed there to the process of vesicle self-reproduction. Herein, we also explain why vesicle budding could have preceded the establishment of cellular life.

![Figure 1. Examples of membrane budding in vesicles with laterally homogeneous membranes observed by optical microscopy. The pictures show vesicle contours of their equatorial cross-sections: a) Limiting shape of the cup shape class. b) Limiting shape with two invaginated buds. c) Limiting shape of the pear shape class (selected from Figures 13, 10 and 5, respectively, of ref. [5]). d) Limiting shape with two evaginated spheres. e) Limiting shape with three evaginated buds (Figures 3 and 1 C, respectively, from ref. [7]). f) An intermediate shape of an originally spherical phospholipid vesicle growing because it is transferred into the suspension of large oleic acid vesicles. At the indicated time, smaller daughter vesicles sprouted from the mother vesicle with an approximately constant rate (taken from Figure 6 of ref. [8].) g) A section of the shape with a large number of connected spheres exhibiting two different radii; the image was obtained by relaxing the tube produced by optical tweezers (taken from Figure 5 c of ref. [9]). h) The limiting shape of the pear class with approximately equal spherical sections (taken from Figure 2 a of ref. [10].)
2. Theoretical Background for the Process of Vesicle Budding

The phenomenon of vesicle budding can be considered as a particular example of the establishment of vesicle shapes. Vesicle shapes can be predicted theoretically by assuming that a membrane with a closed surface attains the shape that corresponds to the minimum of its elastic energy. In his review article on the subject Sackmann stated that "many complex shape transitions of cell membranes can be mimicked by single lipid bilayer vesicles by simply varying the area-to-volume ratio or by chemically induced bending moments, suggesting that these processes are governed by the universal bending energy concept of closed shells composed of stratified membranes". The development of this topic has been extensively reviewed elsewhere. Herein, we will focus on the aspects pertinent to the process of vesicle budding. For the sake of completeness, we will nevertheless give a short account of the relevant contributions to vesicle elastic energy and describe the concept of shape classes and shape trajectories. The emphasis will be on defining the relevant shape determining parameters. Vesicle shapes exhibiting buds will be described as special in that they are the consequence of geometrical constraints.

2.1. Elastic Properties of Stratified Vesicle Membranes

The typical stratified membrane is a phospholipid bilayer composed of two oppositely oriented monolayer leaflets in which the hydrophilic heads of their amphiphilic components face the outer and inner aqueous solutions, while their hydrophobic tails contact in the membrane middle. Despite being in tight contact, the leaflets are free to move past each other in lateral directions. Consequently, they relax their elastic strains in an independent manner. The only constraint is that they are aligned in a parallel manner with a constant distance between their neutral surfaces (h). The area of the neutral surface of the outer leaflet \( A_0 \) is larger than the area of the neutral surface of the inner leaflet \( A_i \) by the integral over the membrane area of the sum of the membrane principal curvatures \( K = \int (C_1 + C_2) \, dA \). The difference between the areas of the two leaflets is thus given by Equation (1):

\[
\Delta A = A_0 - A_i = h \int (C_1 + C_2) \, dA
\]

Integration is over the membrane area of the vesicle A (where, because \( h < A^{1/2} \), \( A - A_i - A_0 \)).

The stretching energy of the bilayer is essentially the sum of the stretching energies of the two leaflets. As the leaflets behave as two dimensional liquids, it can be, for the closed bilayer, expressed as the sum of two independent deformational modes, the area expansion energy term \( (W_A) \), and the nonlocal bending term \( (W_r) \) [Eq. (2)]:

\[
W_A + W_r = \frac{K}{2A_0} (A - A_0)^2 + \frac{k}{2A_0h^2} (\Delta A - \Delta A_0)^2
\]

where \( K \) is the area expansivity modulus (reciprocal of the compressibility modulus), \( A_0 \) is the preferred (i.e. equilibrium) area of the membrane, \( k \) is the nonlocal bending modulus, and \( \Delta A = A_0 - A_0 \) is the difference between the preferred areas of the outer and the inner leaflets \( A_0 \) and \( A_0 \), respectively. The first three constants can also be expressed as functions of the corresponding constants of the bilayer leaflets.

The second term in Equation (2) is characterized as nonlocal because its variable, the area difference \( \Delta A \), is proportional to the integral of the sum of the membrane principal curvatures over the whole membrane area [Eq. (1)]. In comprehending the significance of the nonlocal bending energy term in establishing phospholipid vesicle shapes, it is important to realize that the area difference \( \Delta A \) depends on the shape, whereas the preferred area difference \( \Delta A_0 \) depends on the total amount of molecules that form each leaflet and their areas at neutral surfaces. As in general \( \Delta A \neq \Delta A_0 \), one leaflet is stretched and the other compressed.

The vesicle bending energy can be expressed in terms of principal curvatures and is equal to the integrals of the local bending contributions over the whole membrane area, which are the local bending term \( (W_B) \) and the Gaussian bending term \( (W_C) \) [Eq. (3)]:

\[
W_B + W_C = \frac{1}{2} k_b \int (C_1 + C_2 - C_0)^2 \, dA + k_g \int C_1 C_2 \, dA
\]

where \( k_b \) is the local bending modulus, \( k_g \) is the Gaussian bending modulus, and \( C_0 \) is the spontaneous curvature. Bending modulus and spontaneous curvature are the sums of the corresponding parameters of the two leaflets. The nonzero spontaneous curvature \( C_0 \) reflects membrane asymmetry. An unsupported piece of an asymmetrical membrane would...
assume the mechanical equilibrium at a curved conformation with radius $2/C_p$. Spontaneous curvature as defined in Equation (3) is a local membrane property and depends on the composition of membrane leaflets and intrinsic curvatures of their constituents.

The expression for the elastic energy of the bilayer, that is, the sum of Equations (2) and (3), also holds generally for stratified membranes with more than two leaflets. The area expansivity and local bending constants are in this case the sums of the corresponding moduli of all the leaflets. The nonlocal bending modulus is proportional to the square of the number of leaflets, so that the ratio $k/k_c$ increases as their number increases. In this generalization it is necessary to replace the area difference as the variable in the nonlocal bending term by the integral of the sum of principal curvatures, which in the case of bilayers is already recognized to be $\Delta A/C_2$.

### 2.2. Vesicle Shape Determination and Shape Classes

If a vesicle is in a swelled state, that is, its volume ($V$) is larger than the volume of the sphere with an area of the non-extended membrane $V_s = 4\pi R_s^2/3$, where $R_s = (A_0/4\pi)^{1/3}$ is the radius of the sphere, its elastic energy depends mainly on the area expansion energy term $W_A$ [Eq. (2)]. Under flaccid conditions, that is, when the vesicle volume is smaller than $V_s$, a vesicle attains the shape that is determined primarily by the sum of the local and nonlocal bending energy terms.

The Gaussian term for all shapes of spherical topology assumes the constant value $4\pi k_c$ and can be omitted in shape determinations. It must only be taken into account in processes in which the number of vesicles is changing, as for example in vesicle fusion or fission.

Minimization of the sum of local and nonlocal bending energies must be carried out under the constraints of constant membrane area ($A \approx A_0$) and vesicle volume ($V$). It is convenient to scale the system parameters with the length dimensions relative to the membrane area of the value unity. In the treated the so called generalized bilayer couple model, the relevant parameters that determine the shapes of unsupported flaccid vesicles are then the reduced volume $\nu = V/V_s$, the reduced spontaneous curvature $c_s = C_s R_s$, the reduced preferred difference between the leaflet areas $\Delta a_0 = \Delta A/8\pi h R_s$, and the ratio, $k/k_c$, between the nonlocal and local bending constants. $8\pi h R_s$ is the difference between the areas of the bilayer leaflets for the sphere with radius $R_s$. In order to be more concise, the sum $W = W_A + W_s$ is rewritten in terms of the reduced bending energy (relative to the bending energy of the sphere for zero spontaneous curvature, $w = W/8\pi h k_c$) and reduced curvatures ($c_1 = C_1 R_s$, $c_2 = C_2 R_s$) as [Eq. (4)]:

$$w = w_0 + w_r = \frac{1}{4} \int (c_1 + c_2 - c_0)^2 \, da + \frac{k}{k_c} (\Delta a - \Delta a_0)^2$$

where $da = dA/4\pi R_s^2$, and the reduced difference between leaflet areas $\Delta a = \Delta A/8\pi h R_s$. The variable $\Delta a$ is in its essence geometrical quantity because it is, according to Equation (1), equal to the reduced value of the integral of the sum of principal curvatures over the membrane area [Eq. (5)]:

$$\Delta a = \frac{1}{2} \int (c_1 + c_2) \, da$$

The complete family of possible shapes of the generalized bilayer couple model can be obtained by minimizing the local bending energy for $c_s = 0$ [Eq. (6)]:

$$w_{0,0} = \frac{1}{4} \int (c_1 + c_2)^2 \, da$$

and solving the corresponding equation for all possible values of $\nu$ and $\Delta a$. Depending on the values of $\nu$ and $\Delta a$, the obtained shapes can have different symmetries. The shape classes are defined as the domains in the $\nu$–$\Delta a$ phase diagram, where shapes of the same symmetry can be obtained by continuous shape transformations caused by continuously varying $\nu$ and $\Delta a$. Regions in the central part of the $\nu$–$\Delta a$ shape phase diagram, where the shapes of different classes are shapes with the absolute minimum of $w_{0,0}$, are shown in Figure 3. As also shown there, some but not all shape classes comprise an axisymmetric shape, and some axisymmetric classes also exhibit equatorial mirror symmetry. Class boundaries are either symmetry-breaking lines or the so-called limiting shapes that we shall discuss in Section 2.4.
To find the minimum of Equation (4) for a vesicle with the reduced volume \( v \), it is convenient first to determine the minimum of \( w_{b,0} \) for all values of \( \Delta a \) and then, by inserting the resulting dependence \( w_{b,0}(\Delta a) \) into Equation (4), minimize it with respect to \( \Delta a \) to obtain, by taking into consideration Equation (5), the following equation [Eq. (7)]:

\[
\frac{d w_{b,0}(\Delta a)}{d \Delta a} + 2 \frac{k}{k_c} (\Delta a - \Delta a_0) - c_0 = 0
\]

Equation (7) can then be solved for \( \Delta a \). Among the shape determining parameters \( v \), \( c_0 \), \( \Delta a_0 \) and \( k/k_c \), there are only three independent parameters, because the shapes obtained are the same for any combination of the parameters \( c_0 \), \( \Delta a_0 \) and \( k/k_c \) that give the same value of the sum of the constant terms in Equation (7), expressed for instance as the effective preferred area difference \( \Delta a_0 + (k_c/2k_c)c_0 \). The limit of the generalized bilayer couple model in which the ratio \( k/k_c \) is infinitely large is called the strict bilayer couple model.\(^{16} \) The other limit is the spontaneous curvature model\(^ {40,37} \) in which the ratio \( k/k_c \) is zero.

In the strict bilayer couple model there is the stable shape at any possible set of values of \( v \) and \( \Delta a \). At finite values of the ratio \( k/k_c \), some of these shapes may not be stable. Such stability gaps were found to be the characteristic of the spontaneous curvature model.\(^ {34,37} \) The reason for such behavior is the particular dependence of \( w_{b,0} \) on \( \Delta a \) which is demonstrated in Figure 4a for the disc and cup classes, for the reduced volumes \( v = 0.9 \), 0.6 and 0.3, together with some calculated shape cross-sections. The corresponding \( \Delta a \) dependences of \( d w_{b,0}/d \Delta a \) are shown in Figure 4b. All three examples show the continuous symmetry breaking from the disc to the cup class. The solutions of Equation (7) (Figure 4b) can be obtained graphically as cross-sections of the \( \Delta w_{b,0}/\Delta a \) curve and the line with the negative slope of the magnitude of the ratio \( 2k_c/k_c \). All the shapes predicted by the strict bilayer couple model can be realized as stable shapes if this ratio is so large that this line crosses the \( \Delta w_{b,0}/\Delta a \) curve only once for all values of \( \Delta a_0 \). Below a certain critical ratio \( (k_c/k_c)_c \), these lines cross the \( \Delta w_{b,0}/\Delta a \) curve three times, which indicates discontinuous shape transitions at continuously varying \( \Delta a_0 \). The critical value of the ratio \( k_c/k_c \) defined by the steepest point of each \( \Delta w_{b,0}/\Delta a \) curve (which is in fact at the symmetry breaking point) thus determines whether the system exhibits the behavior of the strict bilayer couple model type or of the spontaneous curvature model type. The dependence of the critical ratios \( (k_c/k_c)_c \) on the reduced volume \( v \) for the cup shapes is presented in Figure 4c. At higher reduced volumes a vesicle may exhibit spontaneous curvature model type behavior and at smaller ones strict bilayer couple behavior. Analogous results were obtained in the region of cigar and pear class shapes.\(^{34} \) Because vesicle shapes within the spontaneous curvature model depend only on the values of \( v \) and \( c_0 \), it is more convenient to use the \( c_0 - v \) phase diagram for the conditions of spontaneous curvature type behavior, instead of \( v - \Delta a \) phase diagram.\(^ {35} \)

### 2.3. Shape Trajectories

The described concepts that underlie vesicle shape behavior have been tested by experimental observations of giant vesi-
cles in which shape trajectories through different shape classes were traced as a result of changes of $\Delta \alpha$, $c_v$, and/or $v$. On continuously varying these parameters, vesicle shape transformations were either continuous or discontinuous. Continuous shape transformations within the class of cup shapes obtained by changing the temperature are shown as an example (Figure 5a)\cite{43}. For comparison, the shapes predicted by the strict bilayer couple model are shown in Figure 5b.\cite{43} These shapes lie within the class of cup shapes on the dashed line plotted in Figure 3 at $v = 0.6$, starting with the discocyte shape (shape 1) and ending with the sphere that contains an invaginated sphere (shape 16). The correspondence between shapes obtained experimentally and those predicted by the theory is rational because, at this reduced volume, the critical ratio ($k_c/k_c^0$) is about 3 and the measured value of the ratio $k_c^0/k_c$ for typical phosphatidylcholine membranes is somewhere between 2 and 3.\cite{44–46} On the other hand, the shape behavior at reduced volumes close to 1 is because of much larger ($k_c/k_c^0$) of the spontaneous curvature type.\cite{47} The shape trajectories observed within prolate (cigar and pear) shape classes were discontinuous, which could be ascribed to the spontaneous curvature type behavior. Analogous tests of bilayer elasticity models of vesicle shapes were also performed by inducing membrane asymmetry by membrane binding compounds.\cite{47}

### 2.4. The Limiting Shapes

Shape trajectories within some shape classes are limited by a budded shape, that is, the shape composed of spheres connected by thin necks. These shapes belong to the so-called limiting shapes that can be predicted as the extremes of the vesicle volume at fixed $\Delta \alpha$.\cite{15, 16} These extremes were shown\cite{48} to consist essentially of a composition of spherical and cylindrical parts where, independently of their number, they may exhibit only two different values of their radii. The limiting shapes of the cup class (Figures 3 and 5B) are combinations of a large and an invaginated small sphere. In the pear class, the limiting shapes comprise two evaginated spheres.\cite{4, 39} Such limiting shapes have the value of the reduced local bending energy $w_b = 2$. As exemplified in Figure 1, a typical example of a more general limiting shape is a larger sphere with a certain number of equal buds. The reduced bending energy of such a budded shape is in case of a symmetrical membrane which does not exhibit a spontaneous curvature equal to the total number of its spherical parts.

Within the strict bilayer couple model, the occurrence of limiting (budded) shapes can be understood to be the consequence of geometrical constraints. This is why these limiting shapes could have been obtained mathematically by the above mentioned variational principle.\cite{44} The limiting shapes lie in the part of the $v – \Delta \alpha$ phase diagram where the shapes are stable for all parameters of the generalized bilayer couple model. Therefore they exist also in its spontaneous curvature model limit. Within this limit it was shown\cite{45} that a vesicle of constant volume and increasing membrane area periodically sheds excess area into a set of smaller spheres with radii comparable to the double reciprocal value of spontaneous curvature. The bending energy of all these shapes is therefore close to zero.

It has to be pointed out that in different corresponding experimental setups, the budding process may not be the property of the whole vesicle but can occur as the consequence of a local perturbation of the membrane. In such cases the buds are not necessarily exactly of the same size (Figures 1f and 3 in ref. [49]) because of the possible influence of dissipative processes.\cite{49, 50}

![Figure 5. Illustration of the shape trajectories: a) Vesicle shape transformations within the class of cup shapes obtained by changing the temperature (Figure 6 of ref. [5]). b) Series of shapes on the corresponding shape trajectory obtained on the basis of the strict bilayer couple model (Figure 1 of ref. [43]). The membrane local bending energy $w_{b,0}$ is given in units of the bending energy of the sphere ($w_{b,0} = W_{b,0}/8\pi k_c$).](image)

### 3. Effects of Membrane Heterogeneity

Erich Sackmann and his colleagues also performed some pioneering studies of vesicle budding by studying the behavior of vesicles with multicomponent membranes.\cite{51, 52} It was demonstrated that the shape transitions into budded shapes can be driven by liquid/gel domain formation and/or coupling of the spontaneous curvature of the
membrane to the local lipid composition. It was indicated that it is either the coupling of composition to morphology which is part of the driving force for the formation of the bud or the requirement for the diminution of the domain boundaries.\textsuperscript{[50]} These concepts were later fully confirmed by the use of high-resolution fluorescence imaging techniques showing directly a correlation between composition of membrane domains and local membrane curvature.\textsuperscript{[54]} An interesting issue in these observations is vesicle fission. While in the case of homogeneous membranes the neck between spherical parts in general remained stable, different mixtures of membrane lipids\textsuperscript{[51,54]} or lipid phase transition\textsuperscript{[49]} could also cause vesicle fission. In principle, the effects of membrane inhomogeneity, due to phase separation of the components of a multicomponent membrane, may overrule the effects of membrane elastic energy. However, in interpreting the budding of these more complex membranes, it has to be kept in mind that there are aspects of this phenomenon that are governed by geometrical constraints.

4. Relation between the Budding Processes in Vesicles and Cells

Many factors suggest that the general features of vesicle shape behavior and the cellular processes that involve membrane budding are related.\textsuperscript{[55]} At the phenomenological level, certain aspects of all these processes can be interpreted on the basis of the understanding of the shape behavior of lipid vesicles. Their similarities could be the consequence of general structural similarities, such as membrane lamellarity, between the two systems. There are for instance consequences of the stratified membrane structure which are independent of structural and compositional details of the constituent leaflets. Membrane budding, which is an obligatory step in vesicle fission and in fusion processes taking place in cellular membrane trafficking, can be often understood on the basis of changes of the preferred area difference \( \Delta A_c \). Cellular constituents, such as phosphoinositides, may by their metabolism affect the parameters \( \Delta A_c \) and \( C_o \) and it may not be a coincidence that they play such an important role in membrane transport.\textsuperscript{[56]} It has been for instance also observed that the compounds that cause changes of the preferred area difference affect the rate of endocytosis.\textsuperscript{[57]}

It can be asserted that the shape behavior of simple vesicular systems can serve at least as a framework for interpreting some aspects of cell morphology. However, cellular processes are deterministic and their performance in general depends on a large number of proteins.\textsuperscript{[58]} All of these proteins may not be absolutely required as was indicated by various reconstitution experiments.\textsuperscript{[56,59]} This notion can be extrapolated towards the assumption that cellular morphogenetic processes emerged from their vesicle counterparts.\textsuperscript{[26]} As budding is the property of the membrane, the proteins which participate in the proper functioning of these processes can be viewed as the result of an evolutionary upgrading to make them controlled and thus more reliable. As an example we shall discuss the process of vesicle self-reproduction as a process from which the phenomenon of the cell cycle could have emerged.

5. Vesicle Self-Reproduction

In the process of its self-reproduction a vesicle must double its volume and membrane area, and split into two daughter vesicles. Vesicle splitting becomes feasible if, during its growth, it transforms its shape from the original sphere into a shape that is a composition of two spheres connected by a narrow neck.

The question can be asked about the shape trajectory from an initially spherical vesicle into the final shape recognized to be the limiting shape of the pear class shapes.\textsuperscript{[48,59]} We approached this problem\textsuperscript{[17]} by considering a growing vesicle suspended in aqueous solution that only contains a minute amount of its membrane constituents, and whose membrane exhibits spontaneous curvature \( C_o \). The shape trajectory could thus be sought in the \( c_o-v \) shape phase diagram of the spontaneous curvature model.\textsuperscript{[37]} Vesicle growth was considered as the consequence of the increase of membrane area due to the continuous incorporation of new molecules into the membrane. The initial shape was assumed to be the sphere with zero bending energy, that is, with radius \( R_0 = 2/C_o \) and area \( A_0(0) = 4\pi R_0^2 \). The reduced variables of this shape are \( v = 1 \) and \( c_o = 2 \). The corresponding values of the end shape of the trajectory with \( V = 2 V_0 \) and \( A_o = 2A_0(0) \) are then \( v = 1/2^{1/2} \) and \( c_o = 2 \times 2^{1/2} \) (Figure 6a). This is so, because the reduced volume is proportional to \( V/V_0^{1/2} \) and the reduced spontaneous curvature to \( A_0^{1/2} \). In the model we assumed that new molecules incorporate into the membrane with a rate proportional to the membrane area, so that the time dependence of the membrane area is exponential [Eq. (8)]:

\[
A_o(t) = A_o(0)2^{vt}
\]

with time \( t \) and area doubling time \( T_o \). The vesicle volume can only change if the membrane is permeable to the solvent, whose net flow is proportional to the pressure difference \( \Delta p \) that is established across the membrane according to its elasticity. Due to water flow through the membrane, the vesicle volume changes with the rate [Eq. (9)]:

\[
\frac{dV}{dt} = L_p A_o \Delta p
\]

where \( L_p \) is the membrane hydraulic conductivity.

At \( L_p = 0 \), a vesicle would be deformed from its spherical shape at the very beginning of its membrane growth. The shape trajectory in such a case could not reach the desired final shape. At finite values of \( L_p \), a vesicle grows as a sphere as long as the pressure difference that drives the net flow of solvent through the membrane is smaller than the critical pressure at which a sphere becomes unstable. This critical pressure for the spontaneous curvature model was obtained analytically by Ou-Yang and Helfrich\textsuperscript{[61]} to be \( \Delta p = \frac{\Delta p}{r^2} \), where \( R \) is the radius of the sphere, and can also be determined numerically by solving the shape equation.\textsuperscript{[19]} It was found that, for the trajectory to reach the self-reproducing shape, the parameters of the system must be interrelated so that the product \( T_o L_p A_o C_o^2 \) assumes a fixed value which, by numerical analysis, was found to be 1.85. The corresponding trajectory in the \( c_o-v \) and \( 2774 \text{ www.chemphyschem.org } © 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim ChemPhysChem 2009, 10, 2769 – 2776
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Figure 6. Two representations of the vesicle shape transformation from a sphere (point A) to the shape which is composed of two equal spheres connected by a narrow neck (point E) (adapted from Figure 1 of ref. [17]). At point B, the vesicle begins to deviate from the spherical shape, point C is the point of the maximum vesicle volume and, at point D, the vesicle exhibits a discontinuous shape transformation from a shape involving equatorial mirror symmetry to a shape without such a symmetry. a) Shape trajectory in the \( c_0 = v \) shape phase diagram. The trajectory (bold line) begins at point A \( (c_0 = 2, v = 1) \) and ends at point E \( (c_0 = 2\sqrt{2}, v = 1/\sqrt{2}) \). The dashed line \( (\Delta p = 0) \) connects points that correspond at each value of \( c_0 \) to maximum values of the bending energy. The line \( D^\text{max} \) is at discontinuous shape transformations from the cigar to the pear shape class and the line \( L^\text{max} \) is the line of the limiting shapes (described in detail in ref. [37]). b) Dependence of the reduced vesicle volume \( \nu \nu \nu \nu \), full line, \( V/V_0 \), and the relative membrane area; dashed line, \( A_i/A_0([0]) \) on the reduced time \( \tau = \tau/T_p \). The axial cross-sections of some characteristic shapes are also presented. Shapes are determined by solving the shape equation.

phase diagram is shown in Figure 6a, and the time dependence of the vesicle volume and the membrane area in Figure 6b, together with the cross-sections of some characteristic shapes. A vesicle grows as a sphere until it reaches the point B \( (c_0 = 2.45) \). The reduced volume then decreases until it reaches the point E. Assuming vesicle splitting, the process continues and gives rise to the growing vesicle population with a doubling time equal to \( T_p \). For values of model parameters for which \( T_p A L \nu C^2 > 1.85 \), the trajectory ends at the line of limiting shapes of the pear shape class \( (L^\text{max} \text{ in Figure 6a}) \), producing daughter vesicles of different radii. In this case the doubling time of vesicle population is larger than \( T_p \).\(^{[17]}\)

We also studied the properties of a model in which we included a solute that can cross the membrane, and looked for the corresponding self-reproducing properties.\(^{[18]}\) In this case, the criterion for self-reproduction became more involved, however, the qualitative features of the behavior of the system remained the same.

The results of vesicle self-reproduction modeling suggest the possibility for the composition of vesicle membrane to evolve. Vesicle self-reproduction can only occur under a condition that relates parameters of membrane mechanics, reflecting membrane composition, and the parameters determining vesicle doubling time that, in addition, reflect external conditions. The relationship obtained thus connects vesicle intrinsic properties and properties of the external medium, which means that it can act as the selectivity criterion in the sense that vesicle populations with shorter average doubling times have better survival advantage than other vesicle populations. The corresponding membrane composition is thus a selected membrane composition, in contrast to all other membrane compositions.

### 6. Outlook

Vesicle shape behavior can be considered as a possible framework for the analysis of cellular processes that exhibit changes of membrane conformations, such as membrane budding. While vesicle properties are describable on the basis of simple physical laws, the corresponding processes in cells are much more complex in that they usually involve large sets of proteins. Full comprehension of the behavior of simple vesicle shapes is necessary at least to avoid ascribing to these proteins the functions for which they are not needed. However, it may also be that the relation between vesicles and cells has roots that are deeper.

Vesicles possess a variety of properties that might have been functional in the evolution of biological systems. By themselves they possess several basic characteristics of the life process and can therefore be thought of as being plausibly involved in the origin of cellular life: they compartmentalize the space; under specific growing conditions they exhibit the phenomenon of vesicle fission which indicates their ability to self-reproduce\(^{[62, 63]}\) by splitting they transmit compositional information\(^{[60]}\) in the sense that their siblings retain the properties of the parent vesicle by inheriting parts of the same internal solution and the same membrane; finally, the existence of the relation between their growth and division parameters indicates a basis for the competitiveness between vesicle populations and the consequent selectivity principle.\(^{[17, 18]}\) Vesicles have also been proposed as an early evolutionary step in the emergence of cellular life on the grounds of the possible abundance of lipids and other amphiphilic molecules in the prebiotic environment.\(^{[65, 66]}\)

It is plausible to expect that, in the course of evolution, all the available properties of molecules and their complexes have been explored. It is therefore tempting to propose that today’s complex life may be an upgraded version of a kind of primitive life process that could have existed based on simple vesicles. The question remains about the possible role of vesicles in further steps of the transition from inanimate to animate state of matter. Because of the lack of records it is, of course, impossible to make progress in understanding such a
transition without speculation. Speculations based on vesicle properties can however be considered to possess some degree of reliability because they depend more on the geometrical constraints of closed membranes and the unique properties of the function \(w_{\text{int}}(\Omega_{\text{def}})\) [Eq. (6)] than on their molecular details and are therefore very general and time-independent. It is for instance reasonable to speculate that accumulation of vesicles with a rather narrow variation in their composition could have served as a matrix for surface mediated metabolic processes which could give rise to better growth conditions. Fusion of vesicles, which would have thus acquired different improvements for their growth, could then lead to a profitable increase of system complexity. Thus, investigations of the process of vesicle budding and splitting in connection to their growth and in combination with top down reconstitution experiments represent a promising basis for studying possible initial steps of the emergence of the cell cycle.

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