The surface aggregation pattern of unbound LDL receptors induced by radially convective diffusion and restricted uniform reinsertion in sites near coated pits: Formal analysis and applications

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We present a formal study aimed to the validation of theoretically obtained results concerning the steady state surface aggregation pattern for unbound Low Density Lipoprotein receptors near coated pits. The receptors will be assumed to move by diffusion and radial convection toward the center of the endocytic traps. Their insertion is assumed to occurr uniformly in restricted annular regions sourroundig the traps. The fundamental properties of the mean capture time and the steady state concentration density of unbound receptors as functions of the involved parameters are formally studied. The implications of the derived results are disscused.

Keywords: Receptor mediated endocytosis; restricted receptor reinsertion; surface distribution

Presentamos un estudio de las propiedades matemáticas de la función de distribución radial de estado estable para receptores libres de lipoproteínas de baja densidad. Dicho estudio se requiere en el proceso de validación de resultados teóricos que caracterizan a los patrones de agregación de los receptores en torno a las trampas endocíticas. El movimiento de los receptores se supone controlado por un proceso de difusión con convección radial dirigida hacia el centro de las trampas. La inserción de los mismos ocurre uniformemente en regiones anulares cercanas a las mismas. El estudio formal de las propiedades de la función distribución de estado estable y del tiempo promedio de captura de los receptores se lleva a cabo considerando la dependencia de estos respecto con los parámetros pertinentes. Se discuten también las implicaciones de los resultados obtenidos sobre el sistema real.

Descriptores: Endocitosis via receptores; reinserción restringida; distribución superficial

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1. Introduction

The low density lipoprotein (LDL) endocytic cycle is the process which permits to the human fibroblastic cells the uptake of cholesterol needed in metabolic processes. The large LDL, particles produced in the liver carry about two thirds of the cholesterol circulating in the bloodstream [16]. Before its internalization, the LDL ligand macromolecule binds to its cell surface receptor. The so formed complexes move in the plane of the cell membrane until they are trapped. This occurs in specialized membrane sites known as coated pits. In a further step, the ligand-receptor units are transported to the interior of the cell in closed vesicles formed by the invagination of the coated pits. At lysosome level the bindings are synthesized. The ligand is retained, and in many experimental situations including the LDL system, the receptor is inserted back in to the cell membrane to perform again their endocytic tasks. This sophisticated internalization process is known as Receptor-Mediated Endocytosis it is used by a number of biologically active molecules, to gain entrance to the cell [17].

Deficiencies in the LDL endocytic cycle are responsible for the ailment known as familial hypercholesterolemia. This is characterized by high levels of circulating LDL and the cholesterol deposition in arteries and tendons [5]. This condition provokes coronary disease and the incidence of strokes. Because of its medical importance the receptor mediated endocytic cycle for LDL particles in human fibroblastic cells has been extensively studied at both experimental and theoretical levels. This has produced a great deal of data and the conceptual framework which permits the mathematical modeling of the process.

A widely accepted assumption considers that the receptors move by diffusion in the plane of the cell membrane [2]. Nevertheless some authors claim that additionally, other mechanisms could influence their transport to coated pits from the sites were they are inserted [4]. An important theoretical problem pertains to the estimation of the rate at which LDL receptors hit coated pits. A faster rate will permit a greater removal of the LDL ligand. Besides diffusion, as a fundamental mechanism for the transport of the LDL receptor, a local radial flow assumed to be produced by the invagination of the coated pits has been invoked [8, 14]. Contrasting the hypothesis of a uniform insertion of the receptors all over the cell membrane in [1], and following experimental results, it was conjectured by Robeneck and Hesz [19] that a preferential insertion of receptors in sites near coated

pits must exist. Furthermore that insertion mechanism would necessarily induce an observable surface aggregation pattern for these particles around the endocytic traps. These surface clusters were called plaques.

If the receptors are inserted in sites close to the endocytic traps it is expected that their mean capture time will be shorter, this independently of their transport device. Preferential insertion was modeled by Wofsy *et. al.* Ref. 21 assuming that the receptors were inserted uniformly in annular regions surrounding coated pits. The effect of this insertion mode on the reduction of their mean capture time was evaluated. In that study it was assumed that the movement of the receptors was controlled solely by diffusion. As a result, preferential insertion is a mechanism which could dramatically reduce the mean capture time based on diffusion and uniform insertion all over the cell membrane. Nevertheless the characterization of the corresponding steady state surface aggregation pattern for the unbound receptors near coated pits was not addressed. We will maintain throughout the term preferential insertion to label an insertion mode similar to the one considered in Ref. 21.

In order to evaluate the effect of radially convective diffusion and a preferential insertion mechanism a suitable model was presented in Ehavarria-Heras [8]. It was concluded that within physiological limits the local radial flow induced by the invagination of the coated pits would have only a negligible effect on the aggregation rate of LDL receptors in these structures. Although this last study contributed in the discussion about the influence of convective transport in the aggregation rate of the receptors in coated pits, the characterization of the surface aggregation patterns for the unbound receptors near coated pits remained as an open problem.

Following the theoretical discussion presented in Ref. 11 Solana *et. al.* [20] introduced a method based on the computer graphics technique of ray tracing [7] to generate the surface aggregation patterns of unbound LDL receptors near coated pits. These patterns were obtained associating a gray tone scale to the values of the steady state concentration function receptors at a radial distance from the centre of a coated pit. In each case black was associated to the maximum possible value for the referred concentration. The study in [20] invoked a radially convective diffusion transport device similar to the one addressed in [8] but considered instead, a generalized radially symmetric insertion mode [10, 11]. In that framework preferential insertion could be included as a particular characterization of the insertion mode. It was concluded that preferential insertion was unable to generate the observed plaques unless the radial flow could take strengths far beyond its expected physiological limit. It was also conjectured that within physiological limits, these plaques could be induced by a continuous and decreasing insertion mode. It is worth to point out that this assumption regarding the form of the insertion mode was not derived from experimental studies as it occurred with preferential insertion. Consequently the further exploration of this paradigm deserves our attention.

In that order of ideas, in this paper considering again the model introduced by [8] and generalized by [20] and assuming the case of a preferential insertion mode we will present the study of the fundamental mathematical properties of the associated steady state concentration function for unbound receptors. The formal study presented here will test the consistency of the analytical methods employed to simulate the surface aggregation patterns reported in [20]. The consequences of the behavior of the aforementioned steady state concentration function on the corresponding mean capture time functional are also presented. This will also contribute to the corroboration of the adequacy of the preferential-insertion radially-convective diffusion model to represent the LDL receptor dynamics. This includes the characterization of the associated surface aggregation patterns. Furthermore, on the basis of the formal analysis presented here we will corroborate in a rigorous way the conclusion that preferential insertion is not an adequate paradigm to induce the observed plaques. Consequently according to Solana *et al* [20] the fact that within physiological limits a continuous and decreasing insertion mode could induce these aggregation patterns leads to the consideration of additional experimental effort aimed to the characterization of the correct insertion mechanism.

The mathematical model is described in Sect. 2. The studies of the properties of the derived steady state surface distribution and the mean capture time functional for unbound LDL appear in Sects. 3 and 4 respectively. For completeness of the presentation in Sect. 5 we will provide the behavior of the mean capture time and the associated surface aggregation patterns for LDL receptors near coated pits. This will be done using in the parameters pertinent to the LDL system in human fibroblastic cells. These results are discussed in Sect. 6.

The present authors corroborate that the surface aggregation patterns reported in Ref. 19 could be explained by diffusion, the radial flow and uniform insertion but only in the case where the flow has strength values beyond its physiological limits. The invoked preferential insertion mode besides diffusion is not capable to induce the reported plaque form surface aggregation patterns. The combination of diffusion, radial convection and preferential insertion of the form considered here could do so but only under suitable flow strength values. For details on the biological background as well as the theoretical framework used to build the model presented here the reader is referred to Refs. 9 and 11.

2. The model

In the experimental system used to study the receptor-mediated cycle for LDL particles in human fibroblastic cells, coated pits cover only 1% of the cell surface and appear to be partially aligned over cellular fibers. Hence the set of coated pits can be approximated by a two dimensional dilute, and ordered system of traps. This induces periodicity properties. If we also invoke symmetry conditions, a simplification could be introduced. As explained in [9] using the Berg-Purcell approximation method [3] we can reduce the real multiple-trap

situation and consider a single trap of radius a surrounded by a circle of radius b. This is calculated according to the surface density of endocytic traps. In the problem studied by Berg and Purcell [3] the particles were supposed to move by the influence of diffusion and were inserted uniformly over the reference annulus $a \leq r \leq b$. The internal boundary at $r = a$ was absorbing and the external at $r = b$ reflecting. The last boundary condition reflects the fact that due to the involved periodicity, on average, it takes the same time for the particle to be trapped by a neighboring sink than what it takes to reach the coated pit closer to the site where it was inserted (see Ref. 9, for a detailed explanation of the rationale of this approximation).

Following [8] we assume here that in the reference annulus $\overline{\Omega} = \{(r,\theta) | a \leq r \leq b\}$ about the trap, a steady state concentration density of particles is maintained by the balance between the number of particles lost to the traps and the number inserted. A radial flow directed toward the center of the traps will be assumed to influence the diffusion transport of the receptors. Also we will assume preferential insertion, *i.e.* the receptors are assumed to be inserted uniformly in annular regions surrounding coated pits [19, 21]. We will also consider that the coated pits has an infinite lifetime. This assumption will correspond to the consideration of the maximum effect of the radial flow on the trapping rate of LDL receptors by coated pits. The study of the receptor mediated endocytic cycle taking into consideration the transient behavior of the traps was studied in Ref. 13. It was found that the infinitely-lived trap model is consistent with experimental data for the rate of aggregation of the LDL receptor in coated pits.

We review now, the radially convective diffusion and preferential insertion model for receptor mediated endocytosis introduced in [8]. The velocity of the membrane flow into the trap is supposed to have the form

$$\vec{V}(r,\theta) = v_r \vec{n}, \tag{1}$$

where $\left|\vec{V}\right|$ has the units of distance/time, v_r is a scalar function of r and $\vec{n}$ is the unit vector pointing out radially from the origin toward (r,θ).

The scalar function v_r must be negative because the flow is towards the trap and $\vec{n}$ points out. Now the amount of membrane A_r passing r per unit time is expressed in terms of v_r by means of

$$A_r = |2\pi r v_r|,$$

for $a \leq r \leq b$. It is has been hypothesized [12] that within experimental conditions the coated pit recycling process maintains a steady state surface distribution. If we want the amount of membrane per unit area to remain constant, then the scalar function A_r must be constant. Hence v_r must have the form

$$v_r = -\frac{\mu}{r}, \tag{2}$$

where the scaling factor μ is a positive constant which we call "flow strength constant". Substituting this expression into Eq. (1) we obtain

$$\vec{V}(r,\theta) = -\frac{\mu}{r}\vec{n}. \tag{3}$$

The equation for the steady state concentration $C(r,\mu)$ of particles at a distance r from the center of the trap moving under the influence of diffusion and radial convection with strength μ is [22],

$$-\text{div}\,(DC) + \text{div}\left(C\vec{V}\right) = S(r), \tag{4}$$

where $D \geq 0$ is the diffusion coefficient of the particle under study, assumed to be a positive constant with units of cm^2/s. and $S(r)$ the rate of particle production in units of particle/$\text{cm}^2\cdot$s, at a distance r from the center of the trap.

To model insertion in plaques [19] following [21] we could assume that $S(r)$ has the form

$$S(r) = \begin{cases} S & a \leq r \leq ma \\ 0 & ma < r \leq b, \end{cases} \tag{5}$$

where S is a positive constant related to the total number of particles inserted.

Substituting Eq. (3) into Eq. (4) one gets that $C(r,\mu)$ satisfies the equation

$$D\nabla^2 C + \frac{\mu}{r}\frac{\partial C}{\partial r} + S(r) = 0. \tag{6}$$

As we already pointed out using the involved periodicity and symmetry properties the single trap approximation can be obtained if we assume that the flux across the boundary at $r = b$ vanishes. This generalizes the Berg Purcell [3] reflecting boundary condition and expresses the proper boundary condition for $C(r,\mu)$ at $r = b$.

The magnitude of the flux vector $\vec{J}$ gives the net number of particles per unit time per unit length crossing a boundary; its units are particle/cm·s. There are two contributions to the flux, one due to diffusion and the second due to the flow. Hence

$$\vec{J} = -D\nabla C + \vec{V}C.$$

Since by radial symmetry the steady state concentration depends only on r, we get that a flux vanishing boundary condition at $r = b$ is equivalent to

$$D\frac{\partial C}{\partial r}\bigg|_{r=b} + \frac{\mu}{b}C(b,\mu) = 0. \tag{7}$$

The absorbing boundary condition at $r = a$ is,

$$C(a,\mu) = 0. \tag{8}$$

Notice that the assumption that $S(r)$ has the form (5) makes it necessary to add continuity conditions: at $r = ma$, *i.e.*,

$$C(r,\mu) \text{ continuous at } r = ma, \tag{9}$$

$$\frac{\partial C(r,\mu)}{\partial r} \text{ continuous at } r = ma. \tag{10}$$

Summarizing, the steady state concentration density $C(r,\mu)$ of particles diffusing on $\overline{\Omega}$ under the additional influence of a radial flow into the trap at $r = a$ with uniform insertion restricted to the region $a \leq r \leq ma \leq b$, an absorbing boundary at $r = a$ and with the flux of particles across the boundary at $r = b$ set to zero, can be modeled by means of Eq. (6) for $S(r)$ as given by Eq. (5) and taking into account the boundary and continuity conditions given by Eqs. (7) through (10). We throughout refer to Eq. (6) and its associated set of boundary and continuity conditions simply as the model.

3. The fundamental properties of $C(r,\mu)$

In this section we obtain $C(r,\mu)$ and establish some of its properties. These will be needed to test the theoretical consistency of the model. This can be also achieved studying the behavior of the associated mean capture time functional. In the next section we will define such functional in terms of the number of unbound receptors averaged over the approximation circle $0 \leq r \leq b$. The required properties of positiveness and monotonicity for the dependence of $\tau(\mu,m)$ on μ will follow from the corresponding ones on $C(r,\mu)$. By proving these properties we will test that the model adequately reproduces the behavior of the real system.

Replacing $\nabla^2 C$ by $1/r\{[\partial/\partial r][r(\partial C/\partial r)]\}$ in Eq. (10) we have that $C(r,\mu)$, under condition (2.5), is given by

$$C(r,\mu) = \begin{cases} K_3 r^{-\mu/D} - \dfrac{Sr^2}{2(\mu+2D)} \\ \qquad\qquad + \dfrac{K_1 D}{\mu} & a \leq r \leq ma \\ K_4 r^{-\mu/D} + \dfrac{K_2 D}{\mu} & ma < r \leq b, \end{cases} \tag{11}$$

where K_1, K_2, K_3 and K_4 are integration constants. Applying the boundary and continuity conditions over $a \leq r \leq b$ we obtain that these constants are,

$$K_1 = \frac{Sa^2m^2}{2D}, \tag{12}$$

$$K_2 = 0, \tag{13}$$

$$K_3 = \frac{a^{\mu/D+2} S\left(2m^2 D + \mu m^2 - \mu\right)}{2\mu(\mu+2D)}, \tag{14}$$

$$K_4 = \frac{S\left(m^2-1\right)a^{(\mu/D)+2}\mu + 2DS\left(1-m^{\mu/D}\right)m^2 a^{(\mu/D)+2}}{2\mu(\mu+2D)} \tag{15}$$

As shown in the next section, to obtain the mean capture time $\tau(\mu,m)$ predicted by the model we need to calculate the number of diffusing particles averaged over the approximation circle $0 \leq r \leq b$. In order to do so, we will need the integral,

$$\int_a^b rC(r,\mu)dr = \frac{DS}{\mu-2D}\left\{\frac{(m^2a^2-a^2)^2}{8D} + b^2a^2\left(\frac{a}{b}\right)^{\mu/D}\left[\frac{(\mu+2D)m^2-\mu-2Dm^{(\mu/D)+2}}{2\mu(\mu+2D)}\right]\right\}, \tag{16}$$

which is valid for $1 \leq m \leq b/a, \mu > 0, D > 0$, and $\mu \neq 2D$, and follows from Eqs. (11)–(15) after some algebraic manipulations. The result can be easily extended to the whole domain $\mu \geq 0$ (see *proposition 4.1*).

To check on the positiveness of $C(r,\mu)$, from Eqs. (6) and (5) using the polar coordinates form for the Laplacian we obtain

$$\frac{\partial}{\partial r}\left[r^{\mu/D}C(r,\mu)\right] = \begin{cases} \left(\dfrac{Sm^2a^2 - Sr^2}{2D}\right)r^{(\mu/D)-1} & a \leq r \leq ma \\ 0 & ma < r \leq b. \end{cases} \tag{17}$$

Hence for $a \leq r \leq ma$ we must have

$$\frac{\partial}{\partial r}\left[r^{\mu/D}C(r,\mu)\right] \geq 0.$$

Consequently $r^{\mu/D}C(r,\mu)$ is increasing and necessarily

$$r^{\mu/D}C(r,\mu) \geq a^{\mu/D}C(a,\mu) = 0.$$

Then

$$C(r,\mu) \geq 0.$$

Similarly for $ma \leq r \leq b$ from Eq. (11) it follows that

$$C(r,\mu) = \frac{\text{constant}}{r^{\mu/D}}.$$

By the continuity of $C(r,\mu)$ at ma, the constant must be positive. Then for $a \leq r \leq b, \mu > 0, D > 0$, we have,

$$C(r,\mu) \geq 0. \tag{18}$$

Using direct algebraic manipulation it is not difficult to demonstrate that there exist a function $\phi(a,m,D,u)$ which does not depend on μ and defined through,

$$\int_a^u f(r,\mu)C(r,\mu)\,dr = \phi(a,m,D,u), \tag{19}$$

where

$$f(r,\mu)=\left(\frac{\mu-D}{uD}-\frac{\mu}{Dr}\right),$$

for $a \leq ma \leq r \leq u \leq b$. Then for $a \leq r \leq u$ and $\mu > 0, D > 0$ we have

$$f(r,\mu) \leq 0, \quad \text{and} \quad \frac{\partial f(r,\mu)}{\partial \mu} \leq 0. \tag{20}$$

Since the partial derivative of $C(r,\mu)$ with respect to μ exists and is bounded, it follows from Eq. (19) that

$$\int_a^u \frac{\partial f}{\partial \mu} C(r,\mu)\, dr + \int_a^u f(\mu,r)\frac{\partial C}{\partial \mu}\, dr = 0. \tag{21}$$

By virtue of inequalities (18) and (20) from the above equation we must have

$$\int_a^u [-f(\mu,r)]\frac{\partial C}{\partial \mu}\, dr \leq 0.$$

Invoking inequality (20) again and considering that the above inequality holds for all u such that $a \leq r \leq u \leq b$ we must have that for $\mu > 0$ and $D > 0$, $C(r,\mu)$ satisfies,

$$\int_a^u \frac{\partial C}{\partial \mu}\, dr \leq 0. \tag{22}$$

Now since $C(r,\mu)$ satisfies Eq. (6), using the polar coordinates form of the Laplacian it can be easily shown that for $a \leq z \leq r \leq u \leq b$, $D > 0$, $\mu > 0$, we have

$$\int_z^u \left[\frac{\partial C(r,\mu)}{\partial r}+\frac{\mu}{D}\frac{C(r,\mu)}{r}\right] dr = R(u,S,m,a,D,z) \tag{23}$$

where

$$R(u,S,m,a,D,z) = \begin{cases} \int_z^u \left(\frac{Sa^2m^2-Sr^2}{2rD}\right) dr & a \leq z \leq r \leq u \leq ma \\ 0 & ma \leq z \leq r \leq u \leq b, \end{cases}$$

Notice that $R(u,S,m,a,D)$ does not depend on μ. Integration in Eq. (23) yields

$$C(u,\mu) - C(z,\mu) + \int_z^u \frac{\mu}{D}\frac{C(r,\mu)}{r} dr = R(u,S,m,a,D,z).$$

Taking the partial derivative with respect to μ in the above equation we have for $a \leq z \leq u \leq b$,

$$\frac{\partial C(u,\mu)}{\partial \mu} - \frac{\partial C(z,\mu)}{\partial \mu} + \int_z^u \left(\frac{C(z,\mu)}{Dr}+\frac{\mu}{Dr}\frac{\partial C(z,\mu)}{\partial \mu}\right) dr = 0. \tag{24}$$

Now define, the real valued function $g(r)$ through,

$$g(r) = \frac{\partial C(r,\mu)}{\partial \mu} \quad a \leq r \leq b.$$

We now show that $g(r)$ is negative for $\mu > 0$ and $a \leq r \leq b$. Suppose firstly without loss of generality that $g(r) > 0$ on a set $P \subseteq \overline{\Omega}$ and assume that $g(r) \leq 0$ on a set A such that $P \cup A = \overline{\Omega}$. We show now that $P = \phi$. Assume that $P = [q,b]$ where $a \leq q$. Since inequality (22) holds in $\overline{\Omega}$ our assumption regarding the sign of $g(r)$ in the interval $[a,q]$ is correct. Since $g(r)$ is continuous, the form of P implies $g(q) = 0$. Now from Eq. (24) we have for $a \leq q \leq u \leq b$.

$$\int_q^u \frac{\mu}{Dr}\frac{\partial C(r,\mu)}{\partial \mu}\, dr = -\left[\frac{\partial C(u,\mu)}{\partial \mu}+\int_q^u \frac{C(r,\mu)}{Dr}\, dr\right]. \tag{25}$$

Since we have chosen $q \leq u \leq b$ then obviously $[q,u] \subseteq P$ and since $\mu/Dr \geq 0$ the left-hand side of Eq. (25) must be positive. Since by virtue of inequality (18) $C(r,\mu) \geq 0$ then the right-hand side of Eq. (25) must be negative. As a conclusion P cannot be taken in the form $P = [q,b]$. In a last possibility we could have $g(r)$ changing signs several times. Without loss of generality lets consider for instance that $P = U_{k=1}^{n} P_k$ for $n \geq 1$ and where $P_k \cap P_j = \phi$, for $k \neq j$. We now show that $P_k = \phi$ for all k such that $1 \leq k \leq n$. In fact, assume that $g(r)$ is positive in a set of the form $P_{k=}[c_k,d_k] \subseteq [a,b]$ then inequality (22) will imply that $g(r)$ is negative in an interval before P_k and also in an interval after P_k and by continuity,

$$\frac{\partial C}{\partial \mu}\Big|_{r=c_k} = \frac{\partial C}{\partial \mu}\Big|_{r=d_k} = 0.$$

Equation (24) will now give

$$\int_{c_k}^{d_k} \frac{\mu}{Dr}\frac{\partial C(r,\mu)}{\partial \mu}\, dr = -\int_{c_k}^{d_k} \frac{C(r,\mu)}{Dr} dr.$$

This is again a contradiction because in the above equation the right-hand side is by virtue of inequality (18) negative and the left-hand side needs to be positive since by our assumption $[c_k,d_k] \subset P$. As a conclusion $P = \phi$. Hence for $a \leq r \leq b$ and $\mu > 0$, $D > 0$, we must have

$$\frac{\partial C(r,\mu)}{\partial \mu} \leq 0. \tag{26}$$

The next result will be needed to study the behavior of the second derivative of $\tau(\mu,m)$ with respect to μ. From Eq. (21) we have

$$2\int_a^u \frac{\partial f(r,\mu)}{\partial \mu}\cdot\frac{\partial C(r,\mu)}{\partial \mu}\, dr + \int_a^u f(\mu,r)\frac{\partial^2 C}{\partial \mu^2}\, dr = 0.$$

Now since inequalities (26) and (20) hold for $a \leq r \leq u$ from the above equation we conclude that

$$\int_a^u |f(r,\mu)|\frac{\partial^2 C}{\partial \mu^2}\, dr \geq 0.$$

This implies

$$\int_a^u \frac{\partial^2 C}{\partial \mu^2}\, dr \geq 0 \tag{27}$$

for $a \leq r \leq u \leq b$.

4. The fundamental properties of $\tau(\mu, m)$

In this section we will state and prove several properties of the mean capture time $\tau(\mu, m)$ for diffusing receptors under radial convection of the form (3) and preferential insertion as shown in (5). We derive first, the explicit form of $\tau(\mu, m)$.

— Definition 4.1. we define $k_{m\mu+}$ as the diffusion convection limited forward rate constant depending on uniform particle insertion over the region $a \leq r \leq ma \leq b$. It is given by

$$k_{m\mu+} = \frac{2\pi Da \left.\dfrac{\partial C(r,\mu)}{\partial r}\right|_{r=a}}{\langle C(r,\mu)\rangle}, \tag{28}$$

where $\langle C(r,\mu)\rangle$ for every fixed value of μ, stands for the number of unbound receptors averaged over the approximation region $0 \leq r \leq b$. It is given by,

$$\langle C(r,\mu)\rangle = \frac{1}{\pi b^2}\int_a^b 2\pi r C(r,\mu)\, dr. \tag{29}$$

We notice that $k_{m\mu+}$ can be interpreted as the flux of diffusing particles into the trap divided by the average concentration $\langle C(r,\mu)\rangle$ or equivalently, as the number of particles hitting the trap per unit time per diffusing particle. Since the steady state assumption implies that the number of particles trapped equates the number inserted into the reference annulus $\overline{\Omega}$, we must have,

$$k_{m\mu+} = \frac{2\pi\int_a^b rS(r)\, dr}{\langle C(r,\mu)\rangle}. \tag{30}$$

— Definition 4.2. Let $\tau(\mu, m)$ be the mean capture time for particles under the assumptions of the model. Then

$$\tau(\mu, m) = \frac{\pi b^2}{k_{m\mu+}}. \tag{31}$$

This definition can be explained straightforwardly. If ρ stands for the trap density on the cell surface, then the product

$$k_{m\mu+}\langle C(r,\mu)\rangle\rho \tag{32}$$

gives the mean number of hits to the trap per unit time per unit area. This quantity equates the ratio

$$\frac{\langle C(r,\mu)\rangle}{\tau(\mu, m)}. \tag{33}$$

The result of Eq. (31) is established by noticing that according to the single trap approximation the outer radius b of the annulus $\overline{\Omega}$ must be obtained from the relation

$$\rho = \frac{1}{\pi b^2}. \tag{34}$$

(See [9] for details).

Using Eqs. (30) and (31) we get for $\tau(\mu, m)$,

$$\tau(\mu, m) = \frac{\int_a^b rC(r,\mu)dr}{\int_a^b rS(r)dr}. \tag{35}$$

Using Eq. (26) we easily conclude that for $\mu > 0$, $D > 0$, $\mu \neq 2D$, and $1 < m \leq b/a$ we have

$$\tau(\mu, m) = \left(\frac{2D}{\mu - 2D}\right)\left\{\frac{m^2a^2 - a^2}{8D} + \frac{b^2}{(m^2-1)}\left(\frac{a}{b}\right)^{\mu/D}\left[\frac{(\mu+2D)\,m^2 - \mu - 2Dm^{(\mu/D)+2}}{2\mu\,(\mu+2D)}\right]\right\}. \tag{36}$$

From Eq. (35) and using inequality (18) it can be easily seen that whenever $m \neq 1$ we have $\tau(\mu, m) \geq 0$. On the other hand From Eq. (36) implies,

$$\lim_{m\to 1}\tau(\mu, m) = b^2\left(\frac{a}{b}\right)^{\mu/D}\lim_{m\to 1}\left[\frac{(\mu+2D)m^2 - \mu - 2Dm^{(\mu/D)+2}}{(m^2-1)2\mu(\mu+2D)}\right] = 0. \tag{37}$$

Hence for the case of $m = 1$, using the result of Eq. (37) we can set $\tau(\mu, 1) = 0$. This will give

$$\tau(\mu, m) \geq 0, \tag{38}$$

for $\mu > 0$ and $1 \leq m \leq b/a$.

For $\tau(\mu, m)$ as given by Eq. (36) we also have,

$$\frac{\partial\tau(\mu, m)}{\partial\mu} \leq 0. \tag{39}$$

To show this, observe that by virtue of Eq. (35) we must have,

$$\frac{\partial\tau(\mu, m)}{\partial\mu} = \frac{2}{(m^2-1)\,a^2S}\int_a^b r\frac{\partial C(r,\mu)}{\partial\mu}\, dr.$$

Hence for $m \neq 1$ inequalities (32) implies the result of inequality (39). If $m = 1$ using the result of Eq. (37) to define $\tau(\mu, 1)$will permit to complete the proof.

Let $\tau(\mu, m)$ be given by Eq. (36) then for $\mu > 0$, and $1 \leq m \leq a/b$ we have,

$$\frac{\partial^2 \tau(\mu, m)}{\partial \mu^2} \geq 0. \tag{40}$$

The result follows from Eq. (35) and inequality (27).

Let τ_{s_m} be the limit of the mean capture time $\tau(\mu, m)$ when μ approaches 0. It is straightforward to show that for fixed m we have

$$\begin{aligned}\tau_{s_m} &= \lim_{\mu \to 0} \tau(\mu, m) \\ &= \frac{b^2 m^2 \log(m)}{2D(m^2-1)} - \frac{2b^2 + (m^2-1)a^2}{8D}.\end{aligned} \tag{41}$$

We notice that when m approaches b/a, τ_{s_m} approaches the mean capture time τ obtained by Berg and Purcell [3], *i.e.*

$$\begin{aligned}\tau &= \lim_{m \to b/a} \tau_{s_m} \\ &= \frac{b^2 (b/a)^2 \log(b/a)}{2D((b/a)^2-1)} - \frac{2b^2 + ((b/a)^2-1)a^2}{8D}.\end{aligned} \tag{42}$$

We summarize the above results in the following,

— *Proposition 4.1. Let* $\tau(\mu, m)$ *be* given by Eq. (36) for $D > 0$, $\mu > 0$, $\mu \neq 2D$, $1 < m \leq b/a$ Then $\tau(\mu, m)$ satisfies the properties (37)-(41) and takes the additional limiting values,

$$\begin{aligned}\lim_{\mu \to 2D} \tau(\mu, m) = \\ \frac{a^2}{624D(m^2-1)} \Bigg\{ \left[312 \log\left(\frac{a}{b}\right) - 256\right] m^2 \\ + \left[192 - 256\log(m) - 156\log\left(\frac{a}{b}\right)\right] m^4 \\ + \left[64 - 156\log\left(\frac{a}{b}\right)\right] \Bigg\}\end{aligned} \tag{43}$$

$$\lim_{\mu \to \infty} \tau(\mu, m) = 0 \tag{44}$$

$$\lim_{D \to 0} \tau(\mu, m) = \frac{m^2 a^2 - a^2}{4\mu} \tag{45}$$

$$\lim_{D \to \infty} \tau(\mu, m) = 0 \tag{46}$$

$$\lim_{m \to b/a} \tau(\mu, m) = \left(\frac{2D}{\mu - 2D}\right) \left\{ \frac{b^2 - a^2}{8D} + \frac{(ab)^2}{(b^2 - a^2)} \left(\frac{a}{b}\right)^{\mu/D} \left[\frac{(\mu + 2D) b^2 - \mu a^2 - 2\left(\frac{b}{a}\right)^{\frac{\mu}{D}} b^2}{2\mu a^2 (\mu + 2D)} \right] \right\} \tag{47}$$

Using these limiting values, $\tau(\mu, m)$, can be extended to a continuous function in the region $D > 0$, $\mu \geq 0$, $1 \leq m \leq b/a$. The proof of these results can be easily obtained.

5. Results

On first hand we briefly review the implications for receptors dynamics derived from the model. In order to do so, we need to characterize the expected physiological value for the flow rate scaling factor μ. The radial flow originates when the portions of the cell membrane associated to coated pits invaginate to internalize the trapped ligand-receptor complexes. Since experimental results [6, 12, 15] indicate that the coated pit has an average lifetime of approximately 5 minutes, we expect that in order to keep the amount of membrane in the neighborhood of the coated pit at a constant level, in that amount of time the flow must transport into the region occupied by the coated pit an amount of membrane equal to its surface area. Since the flux into the trap at $r = a$ due to the flow is $2\pi\mu$ and this corresponds to the net amount of membrane crossing $r = a$ per unit time in the direction of the center of the coated pit our requirement will be satisfied by a flow of strength μ_0 satisfying $10\pi\mu_0 = \pi a^2$, that is, $\mu_0 = (10^{-11})$ cm^2/min. The parameter values [12] for the LDL system are $a = 10^{-5}$cm, $b = 10^{-4}$cm and $D = 2.7 \times 10^{-9}$cm^2/min. Maximally there are 10^5 LDL receptors in the cell surface [18]. The ratio of receptors bound in coated pits to unbound ones [12] is 2.2. Hence for a fixed value of μ Eq. (16) can be used to estimate the constant S in Eq. (5). Using these parameters values Eq. (36) gives $\tau(\mu_0, b/a) = 2.91157$ min. that is, a radial flow that has the physiologically plausible strength μ_0 will have only a negligible effect on the mean capture time τ obtained on the basis of pure diffusion and uniform insertion all over the cell membrane (*cf.* Eq. 42 which gives $\tau = 2.911833$ min).

For a radial flow of strength μ_0 a non-uniform insertion function of the form (5) would require a preferential insertion radius of $3.3a$, in order to halve τ (see Fig. 1). This implies that in the presence of the flow induced by membrane loss as coated pit form vesicles, insertion of receptors must be extremely restricted in order to produce a substantial effect on the mean capture time calculated based on pure diffusion and uniform insertion all over the annulus $\overline{\Omega}$.

Using the computer graphics technique of ray tracing [7] we can reproduce the corresponding expected steady state aggregation pattern for LDL receptors near coated pits. To do so, for each μ fixed we rotate the plot of the concentration function $C(r, \mu)$ with respect to an imaginary axis through $r = 0$ and perpendicular to the interval $a \leq r \leq b$. Next, for the so generated surface $z = C(r, \mu)$ the value of z will correspond to a level of gray proportional to the value which $C(r, \mu)$ takes on a circle of radius r, for every value of μ.

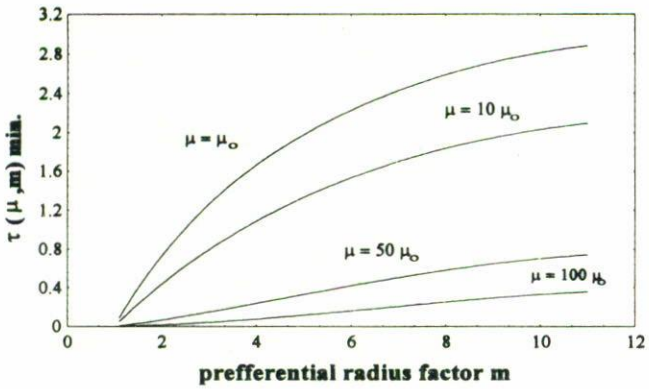

FIGURE 1. The potential of the preferential insertion mode (5) to reduce the mean capture time τ calculated on the basis of diffusion and uniform insertion all over the cell membrane. We observe that for a flow of strength μ_0 halving τ requires a plaque radius of 3.3a.

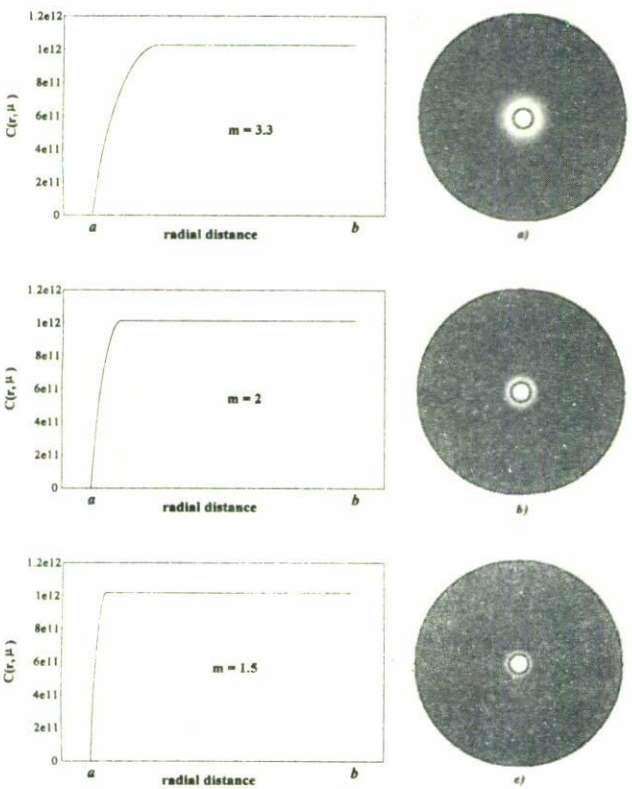

FIGURE 2. The steady state surface aggregation pattern for unbound LDL receptor near coated pits induced by diffusion and a preferential insertion mode of the form given by Eq. (5). Different values of the insertion radius ma were considered. The smaller the value of m smaller the depletion region for the concentration of unbound receptors about coated pits (see *c*). This gives an idea of the effectiveness of the preferential insertion mechanism to enhance the trapping rate induced by diffusion and uniform insertion. Nevertheless assuming diffusion, the sole action of this mechanism fails to induce the reported surface plaque form receptor aggregation patterns.

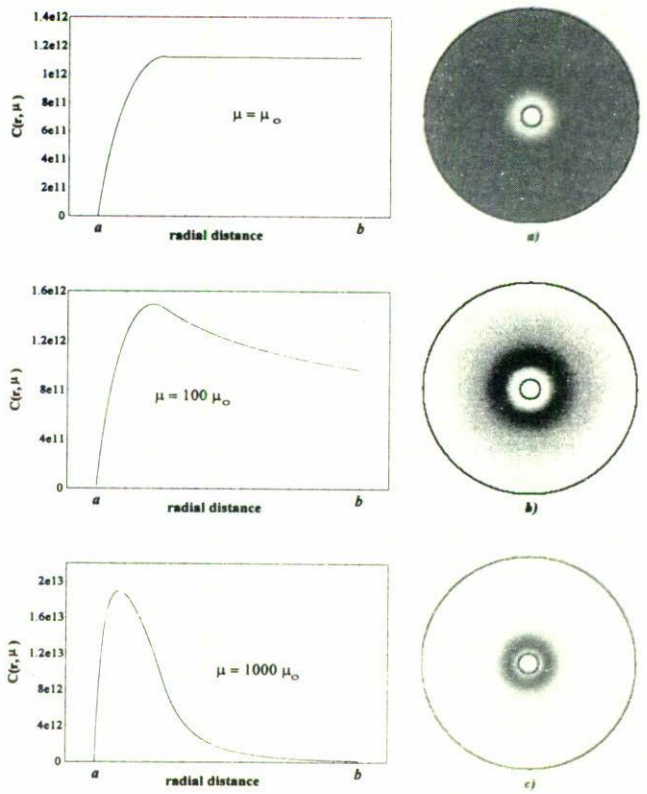

FIGURE 3. The combination of diffusion, preferential insertion and radial convection can induce the reported surface aggregation patterns for LDL receptors. (See Ref. 19). This will occur only for values of the flow strength μ of the order of $100\mu_0$. For that value a plaque begins to form. (see b). A clearly depicted plaque is shown for $\mu = 1000\mu_0$. (See c). To produce these figures a preferential insertion radius $m = 3.3a$ was considered.

Black will be associated to the maximum value of $C(r, \mu)$. The projection of the so constructed surface over the annulus $\overline{\Omega}$ will simulate the expected aggregation pattern.

The preferential insertion mechanism unaided by the radial flow fails to produce the observed annular clustering of the LDL receptors surrounding, coated pits called plaques [19] (see Fig. 2). Furthermore, the diffusion, radial flow and preferential insertion mechanism will fail to produce the plaque effect unless μ takes extremely high values. Figure 3 shows an example obtained for $m = 3.3$ and different values of μ. We can observe that a surface aggregation pattern similar to the reported plaques will form only if μ takes values far beyond the physiological expected value μ_0. It is shown in Fig. 3c, that the plaques reported by Robeneck and Hesz [19] will show up for $\mu \geq 100\mu_0$.

6. Conclusions

Proposition 4.1 summarizes the behavior of the mean capture time $\tau(\mu, m)$ for diffusing particles inserted uniformly into a region of $a \leq r \leq ma$ about a trap of radius a, and moving under the additional influence of a radial flow with con-

stant strength μ [*cf.* Eq. (2)] and directed toward the center of the trap. For instance, as a function of μ, $\tau(\mu, m)$ decreases monotonically and its graph does not have inflection points. The theoretical exploration of the dependence of $\tau(\mu, m)$ with respect to m can be easily obtained as well. We conclude that $\tau(\mu, m)$ as given by Eq. (36) possesses all the desired properties to model the behavior of the mean capture time for diffusing and drifting LDL receptors among the periodic set of endocytic traps. Consequently the formal analysis summarized in *proposition 4.1* permits to conclude that the model of Eq. (6) and its associated set of boundary and continuity conditions exhibits the theoretical consistency required to model the receptor mediated endocytic cycle that we have addressed. Hence the aggregation patterns induced by $C(r, \mu)$ are expected to resemble the clusters that under physiological conditions must be observed in the experiments.

The invoked receptor transport and insertion mechanism will in fact reduce the mean capture time τ (see Eq. 42) for suitable values of m and the expected value of μ. Nevertheless, the device will fail to produce a plaque form surface aggregation pattern unless μ takes extremely high values. The ability of the cell to sort the recycled LDL receptors to different targets within the cell or its surface has been reported [17]. This conceptual framework permits to consider a generalized form for $S(r)$. As its has been shown by Echavarría and Solana [10, 11] a continuously decreasing insertion rate function acting along with diffusion proved to be a more efficient mechanism than a step function of the form (5) for the reduction of the mean capture time τ. At a theroretical level, the results reported in [20] extend the conclusions of the present study. According to these findings a continuous and decreasing insertion mode could generate within physiological limits the observed aggregation patterns. Nevertheless, the existence of a particular form for the insertion rate function with these features remains an open research question. Particularly more experimental exploration could elucidate the matter.

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1. R.G.W. Anderson, M.S. Brown, and J.L. Goldstein, *J. Cell Biol.*, **10** (1977) 351.
2. L.S. Barak and W.W. Webb, *J. Cell Biol* **95** (1982) 846.
3. H.C. Berg and E.M. Purcell, *Biophys J.* **20** (1977) 193.
4. M.S. Bretscher, *Science* **224** (1984) 681.
5. M.S. Brown and J.L. Goldstein, *Proc. Natl. Acad. Sci. USA* **71** (1974) 788.
6. M.S. Brown and J.L. Goldstein, *Cell* **9** (1976) 663.
7. J.W. Dowell, *Graphical Ray Tracing: An anvanced text*, (Chan Street eds, El Segundo CA, 1973).
8. H. Echavarría-Heras, *Mathematical Biosciences* **89** (1988) 9.
9. H. Echavarría-Heras and E. Solana-Arellano, *Miscelanea Matemática* **20** (1994) 28.
10. H. Echavarría-H and E.Solana-Arellano, *Aportaciones matematicas, serie comunicaciones* **18** (1996) 87.
11. H. Echavarría-H and E.Solana-Arellano, *Rev. Mex. Fís.* **42** (1996) 790.
12. B. Goldstein, C. Wofsy, and G. Bell, *Proc. natl. Acad. Sci. USA* **78** (1981) 5695.
13. B. Goldstein, R. Griego, and Wofsy C., *Biophys. J.* **46** (1984) 573.
14. B. Goldstein, C. Wofsy, and H. Echavarria, *Biophysics* **53** (1988) 405.
15. J.L. Godlstein, S.K. Basu, G.Y. Brunschede, and M.S. Brown, *Cell* **7** (1976) 85.
16. J.L. Goldstein, R.G.W. Anderson, and M.S. Brown, *Nature* **279** (1979) 670.
17. J.J. Linderman and D.A. Lauffenburger, *Receptor/Ligand sorting along the endocytic Pathway*, (Springer-Verlag, Berlin Hedelberg, 1989).
18. R.E. Pitas, T.L. Innerarity, K.S. Arnold, and R.W. Mahley, *Proc. Natl. Acad. Sci. USA* **77** (1979) 285.
19. H. Robeneck and A. Hesz, *European J. Cell Biology* **31** (1983) 275.
20. E. Solana-Arellano, H. Echavarria-Heras, and C. Leal-Ramirez, *IMA Journal of mathematic applied in Medicine and Biology* **15** (1998) 1.
21. C. Wofsy, H. Echavarría-Heras, and B. Goldstein, *Biophys* **7** (1985) 197.
22. Stakgold Ivar, *Green's functions and boundary value problems*, (John Wiley & Sons Inc. , New York, 1979) p 7.