Revista Mexicana de Física **42**, *No. 5 (1996) 790–809*

The effect of a general radial reinsertion function in the aggregation rate of low density lipoproteins receptors in coated pits: A theoretical evaluation

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Recibido el 7 de agosto de 1995; aceptado el 11 de marzo de 1996

ABSTRACT. Using the Berg-Purcell approximation we conceive a mathematical model to estimate the effect of a general radially dependent and symmetric reinsertion rate function of LDL receptors near coated pits. The model is proposed as a tool to estimate the potential effect of a the non uniform insertion mechanism on the reduction of the mean capture time of LDL receptors by coated pits. The functional which gives its dependence on the insertion rate function is derived. Its use in particular characterizations of the reinsertion rate function is illustrated. The model provides a criteria to test the assumption of fundamental control of diffusion for receptor movement in the plane of the cell membrane.

RESUMEN. Usando la aproximación de Berg y Purcell, se concibe un modelo matemático para estudiar el efecto de un modo de inserción radial y simétrico para receptores LDL en regiones cercanas a las estructuras de absorción en la endocitosis vía receptor. El modelo permite evaluar el efecto inducido por cualquier modo de inserción no uniforme en el tiempo promedio de captura de los receptores. La funcional que lo expresa en términos del modo de inserción se proporciona en forma explícita. El uso de esta se ilustra mediante caracterizaciones particulares de la función de inserción. El modelo permite evaluar la hipótesis de control fundamental por difusión para el movimiento de los receptores en la membrana celular.

PACS: 87.10; 87.22.F; 87.15.K

1. INTRODUCTION

Selective internalization of biologically relevant macromolecules, too big for cellular pores, channels or simple carriers, require the use of specialized membrane receptors. This process is known as receptor mediated endocytosis.

The specialized membrane structures where the internalization occurs are known as coated pits. They have been observed on the surface of almost all cell except erythrocytes [17]. In electron micrographs appear as fuzzy areas composed predominately of clathrin [36]. The coated pits inv aginate to form closed vesicles which bring to the interior of the cell the needed molecular bindings, also called ligand-receptor complexes.

The purposes of the internalization of extracellular molecules are diverse. Some molecules, *e.g.*, asialogycoproteins, or immune complexes are internalized for the purpose

of removing them from the extracellular medium. Nutrients such as iron or substances like cholesterol are internalized for cell function.

The cell membrane receptors have a signal transduction function. This is why some hormones growth factors or other effector molecules activate only when they are internalized bound to surface receptors. This includes some pharmacological agents. Furthermore even viruses and toxins also gain entrance to the cell via the receptor mediated endocytic cycle [31]. This is why it has been the subject of intense experimental research and modeling.

After the aggregation of the bind complexes in coated pits followed by their internalization by invagination, receptor mediated endocytosis includes a stage where the complexes are synthesized, separating the ligand molecules from their receptors. The ligands are retained for cell function. In some cases the released receptors are reinserted back to the membrane to perpetrate again molecular aggregations. This will be elements of further internalizations. Examples of reinsertion of receptors have been documented for low density lipoproteins (LDL) [4, 10], transferring [6, 27, 30], asialoglycoproteins [45, 43], α_2–macroglobulin [28,46] and insulin [32].

This paper will be focusing on aspects of the dynamics of the receptors associated to the endocytic cycle for LDL particles in human fibroblastic cells. For this process coated pits and their associated receptors have been studied most extensively in cells grown in culture [8], being the LDL receptor the one for which more experimental research has been conducted [10]. The large LDL particles are cholesterol carrying macromolecules produced in the liver and circulating in the plasma. Once the bound complexes are internalized by coated pits they are degraded, and the cholesterol so released serves as the main source of cellular cholesterol. One of the reasons why the LDL endocytic cycle has been so extensively studied is its important role in the process of removal of cholesterol from the plasma. Deficiencies in this process are supposed to be responsible for the ailment known as familial hipercholesterolemia. It is characterized by high levels of circulating LDL. Its onset promotes atheroesclerosis, a previous condition for the occurrence of strokes and coronary disease [23]. It is considered [9], that a severely depleted number of LDL receptors promotes high levels of circulating cholesterol because LDL internalization requires receptor binding. Research efforts to understand the cholesterol uptake process must necessarily include the study of LDL receptor dynamics.

The LDL receptor contains in its structure multiple copies of a 40–aminoacid, cystene sequence. This sequences have also been observed in the precursor for epidermal growth factor (EGF). Furthermore one half of the aminoacids in the human LDL receptor are homologous to a 400-amino-acid region in the human EGF precursor [18]. This raises the claim that the LDL receptor also plays a relevant role in the control of cellular growth processes.

In this paper we focus on the role of the LDL receptor in the cholesterol metabolic process. Particularly in the estimation of the rate at which receptors for LDL particles hit coated pits in human fibroblastic cells. This has been an important biophysical research problem. Some researchers [2] claim that receptors are inserted uniformly over the cell membrane. In other assumption, they are inserted preferentially in restricted areas surrounding coated pits [38]. Keizer *et al.* [29], claim that if reinsertion is uniform then under diffusion control the steady state radial distribution function of receptors around coated

pits must be increasing. The theoretical problem that we are addressing here pertains to the characterization of the steady state distribution function of unbound LDL receptors near coated pits. We will consider the general situation where the insertion rate mode is an arbitrary symmetric and radially dependent function. A direct application of our results will permit the estimation of the effect of the assumed reinsertion rate function, on the mean capture time of LDL receptors by coated pits.

We will conclude that an aggregated and uniform distribution of LDL receptors around coated pits as envisioned by Robeneck and Hesz [38] is incompatible with the hypothesis of fundamental diffusion control for the movement of LDL receptors in the plane of the cell membrane. It will be also concluded that although this hypothesized preferential insertion reduces the mean capture time of LDL receptors by coated pits, it could be a less effective mechanism in comparison to a particular continuously decreasing insertion mode. In any event reinsertion must be very restricted to enhance the refereed trapping rate.

In Sect. 2 we present the biological conceptual model for the receptor mediated endocytic cycle for LDL particles in a human fibroblastic cell. The theoretical methods are presented in Sect. 3. There we describe the Berg-Purcell approximation device. This permits to replace the real multitrap problem by a single circular trap surrounded by a properly chosen annular region where particles diffuse between an absorbing and a reflecting boundary. Section 4 presents previous successful applications of the theoretical methods used here. In Sect. 5 we discuss the simplifying assumptions which will make it possible, to build a tractable mathematical model to estimate the rate at which the inserted and diffusing particles hit the traps. The model for a general reinsertion mode is presented in Sect. 6. The derived mean capture time is presented in Sect. 7. A discussion which evaluates the Robeneck and Hezs [38] assumption of preferential reinsertion in contraposition to the theoretically obtained form for the steady state concentration $C(r)$ of unbound receptors appears in Sect. 8. An appendix contains derivations of the results. For detailed proofs of these and others related calculations the reader is refereed to Ref. [14].

2. THE CONCEPTUAL MODEL OF ANDERSON, GOLDSTEIN AND BROWN

The LDL particles carry about two-thirds of the cholesterol in human plasma (for a review of the physical structure of LDL see Ref. [23]). The receptor appears to be a single, negatively charged, glycoprotein chain [40]. Maximally there are 20,000 to 100,000 LDL receptors on normal human fibroblasts [11, 37]. When LDL is internalized by human fibroblasts, it is eventually brought to lysosomes and degraded, freeing about 1500 cholesterol molecules per LDL particle. The freed cholesterol is used by the cell for plasma membrane synthesis. It also regulates the cell's free cholesterol content in several ways. It reduces the activity of the enzyme *3-hydroxy-3-methylglutaryl coenzyme-A* reductase (HMG-CoA reductase) which is needed for the cell's own synthesis of cholesterol and it stimulates the activity of the enzyme acylCoa:cholesterol acyltransferase, which catalyzes the reesterification of the free cholesterol for storage. LDL also inhibits the synthesis of LDL receptors, thus reducing the amount of additional cholesterol brought into the cell by LDL internalization and degradation [24, 23].

A number of mutant human fibroblasts have been identified which result in impaired LDL receptor function and consequently, inefficient LDL internalization. The disease state associated with such genetic impairment is called familial hypercholesterolemia (FH). It is characterized by high levels of plasma LDL, deposition of cholesterol in arteries and tendons, and early onset of heart disease. In the homozygous form of receptor-negative FH, fibroblasts fail to bind and take LDL via the LDL receptor [13, 26, 24, 3]. A second class of mutant fibroblasts has been observed in which specific LDL binding occurs, but is reduced to 25% or less of normal [25]. A third and much more rare from of FH has been detected where the LDL receptors bind LDL normally, but are incapable of being trapped in coated pits [12]. All of these mutants have been used to help delineate the LDL pathway on human fibroblasts [23].

An experimental arrangement to study the receptor mediated cycle for LDL, is obtained by adding ferritin-cojugated LDL to human fibroblasts at a temperature of 4°C. At this temperature the cell binds LDL to coated pits but do not undergo endocytosis [7]. Observations under electron microscopy of thin section of these labeled cells reveal that about two thirds of LDL-ferritin is attached to coated pits. Considering that coated pits account for only 2% of cell surface, it is believed, that receptor-LDL binding occur in coated pits. When the labeled cells are warmed to 37°C for a few minutes, much of the ferritin is found in membrane coated vesicles or in smooth vesicles inside the cell. At times closer to 30 minutes after the rise of the cell temperature to 37°C the LDL is found at the lizozomes. At this level of observation, it is also noticed, that the LDL receptor is returned to the cell surface to be reutilized. Based on these observations a model [2] for the LDL endocytic-cycle in human fibrolasts has been proposed. It firstly considers the aggregation of the ligand-receptor complexes in coated pits. Afterwards, the coated pit buds into the cell yielding a coated vesicle. Next, the vesicle sheds its coat the LDL is released from its receptor, the receptor is returned to the cell surface to start further endocytic cycles and the LDL particle transported to the lysozomes where it is degraded. This model is shown in Fig. 1. Our research will use this conceptual model as a general framework for mathematical modeling.

3. THE BERG-PURCELL APPROXIMATION METHOD

Using the model to be developed, we will calculate the rate at which diffusing particles (receptors) hit traps (coated pits) on a two-dimensional surface (the cell). This rate will be denoted by k_+ and it is also known as the diffusion limited forward rate constant. It gives the flux in to the trap per unit time per diffusing particle. It is connected to the mean time for a particle to hit a trap (mean capture time) through the relation

$$\tau = \frac{1}{k_+ P} \tag{1}$$

where P is the trap density.

In the two dimensional case k_+ is defined for a circular sink of radius a by means of the equation

$$k_+ = \frac{2\pi a}{\langle C \rangle} \frac{\partial C}{\partial r}, \tag{2}$$

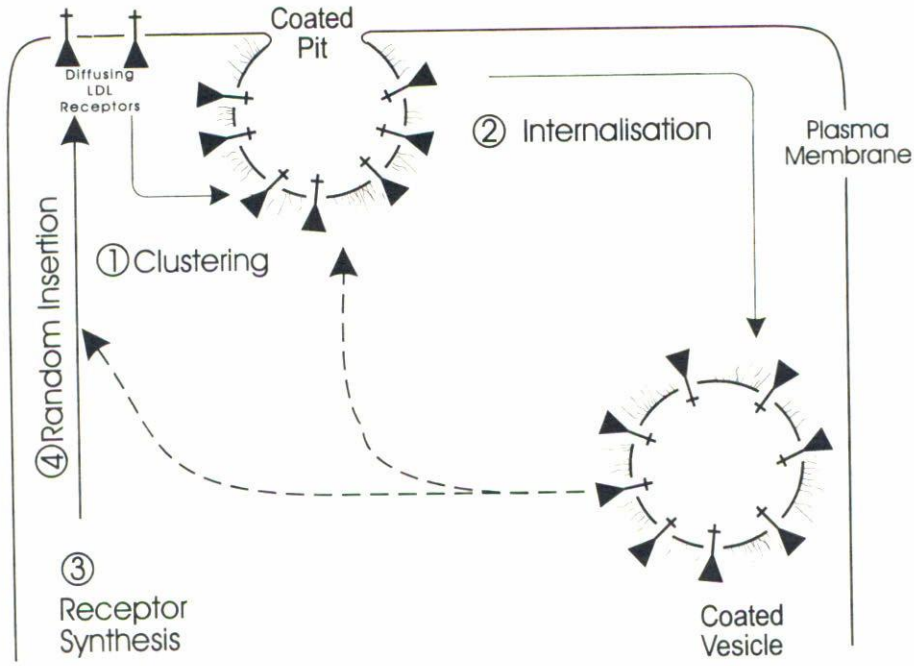

FIGURE 1. The conceptual model of Anderson Goldstein and Brown for the receptor mediated endocytic cycle. For a detailed description of the involved stages, see Sect. 2.

where the derivative is evaluated at $r = a$ and $\langle C \rangle$, is the receptor concentration averaged all over the diffusion space.

In three dimensions, Smoluchowski [44] calculated k_+ for an infinitely dilute system of traps. He solved the steady-state diffusion equation for $C(r)$ subject to the boundary conditions that $C(r)$ vanished at the surface of the trap, and was equal to a positive constant c_∞ at infinity. He then obtained k_+ by calculating the flux into the trap, divided by c_∞. However, in two dimensions there is no solution to the steady-state diffusion equation with these boundary conditions.

The Berg-Purcell [5] approach is an alternate way to get around this problem. It can be thought of as an approximate way to treat ordered systems of traps. As a background for this approximation we can cite a previous calculation. Adam and Delbrück [1], obtained the mean time until a ligand diffusing on a cell surface in a disk of radius b encounters a stationary circular receptor of radius a at the center of the disk. They found that if b is much larger than a, then the mean hitting time τ_1 is given approximately by

$$\tau_1 = \frac{b^2}{2D}\left(\ln\left(\frac{b}{a}\right) - \frac{1}{2}\right),$$

where D is the constant two-dimensional diffusion coefficient.

Using the Berg and Purcell approximation the mean hitting time τ for particles starting at random locations and diffusing in the same annular region considered by Adam and

Delbrück, is found to be

$$\tau = \frac{b^4 \log\left(\frac{b}{a}\right)}{2D(b^2 - a^2)} - \frac{3b^2 - a^2}{8D}. \tag{3}$$

In the limiting case considered by Adam and Delbrück, when $b \gg a$, τ_1 and τ agree to highest order.

The biological setting in which Berg and Purcell were interested in, concerned the interaction of diffusing ligands with stationary receptors distributed on the cell surface. The mean capture time for a ligand was then the mean time until the ligand was trapped in one of many absorbing regions. The single-trap problem they solved approximated the many-trap problem of interest through an appropriate choice of the size of the region considered and through appropriate boundary conditions. Specifically they assumed that:

1. A circular sink of radius a, the radius of a single trap, is located at the center of a circle of radius b satisfying

$$N(\pi b^2) = A, \tag{4}$$

where N is the number of absorbers on the cell surface and A is the surface area of the cell.

2. Diffusing particles start at random locations in the ring of inner radius a and outer radius b about the trap.
3. The mean absorption time $w(r)$ for a particle starting at a distance r from the center of the absorber where $a \leq r \leq b$, satisfies the equation

$$\nabla^2 w + 1 = 0,$$

with the absorbing boundary condition

$$w(a) = 0,$$

and the reflecting boundary condition

$$\left.\frac{\partial w}{\partial r}\right|_{r=b} = 0.$$

The reflecting boundary condition provides an appropriate simplification to the true many-absorber problem in which the ligand can diffuse away from one sink and be trapped by another. We can explain its rationale in the following way:

Let us consider an ordered system of traps. Suppose that we have associated to each trap its share of the cell surface area. This can be achieved using Eq. (4) assigning a reference disk of radius b to each trap of radius a. Lets consider a particle that has been reinserted in a particular annulus surrounding a trap. It wanders around for some time until it reaches the trap or it diffuses away reaching the boundary of the reference disk. If this happens the involved trap periodicity will imply that instead of leaving the particular

reference disk and diffuse to a neighboring one, it will be equivalent for the particle to be reflected at the boundary and diffuse back to the first absorber.

These ideas can be used to model the receptor-mediated endocytosis process by considering the coated pit to be a circular absorber of radius a surrounded by a circle of radius b calculated using Eq. (4) and then assuming that the receptors diffuse in the annulus about the absorber. Instead of calculating the mean capture time directly as Berg and Purcell proceeded, we will obtain it, by means of Eqs. (1) and (2) solving the equivalent problem of the determination of the associated radially symmetric concentration $C(r)$ of diffusing particles. Echavarría and Solana [15] present a detailed discussion of the adaptation of the ideas of Adam and Delbrück and Berg and Purcell to the modeling of the receptor mediated Endocytosis process for LDL particles. In the case where insertion of recycled receptor occurs near coated pits, we deal with the local dynamics of particles diffusing around traps. The Berg Purcell approximation method is in this case a reasonable simplifying device.

4. PRELIMINARY MATHEMATICAL MODELS

Goldstein, Wofsy and Bell [21] and Goldstein, Griego and Wofsy [20] used the Berg-Purcell approximation to calculate k_+ for models based on generalizations of the conceptual model of Anderson, Goldstein and Brown regarding coated pit behavior. They assumed that a steady state concentration of coated pits is maintained by a recycling process. One of the questions they addressed was whether or not the random insertion of LDL receptors into the plasma membrane followed by pure diffusion with the diffusion coefficient measured for LDL receptors could give rapid enough aggregation of LDL receptors in coated pits in order to account for the observed rate of removal of LDL from the cell surface. Since an experimentally determined lower bound for the forward rate constant k_+ is $2.3\times10^{-10}\pm1.6\times10^{-10}$ cm^2/s and the value obtained by these authors is 1.9×10^{-10} cm^2/s, the answer to the question is that the hypothesis of random insertion and pure diffusion of LDL receptors to coated pits is consistent with experimental observations but just barely. Hence, the experiments do not rule out the possibility that the true rate of accumulation of receptors in coated pits could be actually faster than predicted on the basis of random insertion of receptors followed by pure diffusion to coated pits. For instance, if we calculated the rate at which receptors hit coated pits assuming that receptors move solely by diffusion, when in addition we assume convection, we might expect a different value [7]. Also, if receptors are reinserted in restricted areas close to coated pits [38], intuitively the hitting rate will be faster than the one calculated based on uniform reinsertion all over the cell membrane.

Echavarría Heras [16], addressed the theoretical problem of estimating the effect of both; a radial flow directed to the center of the trap and a transverse flow in the plane of the cell membrane. The radial flow originates when the portions of the cell membrane associated to coated pits invaginate to internalize the trapped ligand-receptor complexes. Bretscher [7] suggests the existence of a transverse flow of particular membrane components across the cell membrane. He discusses experiments with motile fibroblasts which suggest that the parts of the cell membrane, internalized by coated pits are returned to the leading edge of the cell. This initiates a bulk flow of lipids and receptors away from the

front of cell. This flow contributes to the transport of large molecular aggregates on the cell surface, from the front to the rear of the cell generating a process known as capping. Echavarría Heras [16] concludes that within physiological limits these flows will have a negligible effect on the rate at which diffusing receptors hit coated pits. Wofsy, Echavarría and Goldstein [47] calculated the effect of preferential insertion of receptors in a particular situation where the insertion rate is a step function. They found that unless this preferential reinsertion is very restricted it will not enhance the trapping rate. Goldstein, Wofsy and Echavarría [2] considered models for receptors moving by diffusion and convection among transient and disordered traps. They found that the models introduced by Echavarría Heras [16], assuming an ordered and stationary system of traps; give good approximations for the system analyzed. This shows that the assumption of an ordered system of sinks not only makes the mathematical problem tractable but also provides, through the ideas of Berg and Purcell a reasonable approximation for the experimental system analyzed. In this vein the results of Keizer *et al.* [29] corroborate our claim. For an updated account of the receptor mediated endocytic process and its mathematical modeling the reader is referred to Ref. [31].

5. MODELLING ASSUMPTIONS

In order to conceive a mathematical model for the receptor mediated endocytiosis process for LDL in human fibroblastic cell, we will need to depend on assumptions derived from the conceptual model of Anderson, Goldstein and Brown and from experimental results obtained by other cell biologists. We will also depend on the approximation method presented in section three for systems of dilute and ordered sinks in a two dimensional environment of diffusing particles.

On cultured human fibroblasts, receptors for certain ligands (*e.g.* insulin, epidermal growth factor and α_2–macroglobulin) cluster in coated pits only after exposure to the ligand [42, 33], while receptors for LDL, cluster in coated pits independently of ligand binding [2]. This feature of the LDL receptor pathway makes it a particularly attractive candidate for mathematical modeling since initially we can ignore the details of the ligand-receptor interaction and still study the recycling of the receptor and its interaction with the coated pit.

It is generally thought that the maintenance of receptors on the cell surface is due primarily to receptor recycling rather than to de novo synthesis. The evidence comes from experiments with cycloheximide to block protein synthesis. In this experimental setup it is observed that the number of LDL receptors in the cell surface remain roughly constant for at least six hours [2]. Evidence of receptor internalization and reinsertion in unblocked system would support the assumption that a steady state concentration of receptors is maintained at the cell surface. Bretscher [7], argues that the time the receptors spent in the interior of the cell is negligible. The basis of his claim is the apparent undetectable pool of receptors (reported by Basu *et al.* [4]) inside the cell during the endocytyc process. He calculates the transit time for an LDL receptor from its binding on coated pits to its reappearance in the membrane and founds it to be of the order of 15 seconds. Based on these ideas we will abide to the general assumption that the internalization and recycling

of LDL receptors during the LDL endocytic process in human fibroblastic cells maintains an steady state cell surface concentrations of receptors.

The set of coated pits will be considered as a diluted and ordered system of traps. The traps are taken to be dilute because on human fibroblasts coated pits cover 1% of the cell surface at 37°C. (Coated pits cover 2% of the cell surface at 4°C [3,35], but when the temperature is raised to 37°C the number of coated pits on the surface is reduced by half [2].) Coated pits appear to be partially ordered on human fibroblasts. They tend to be linearly aligned over intracellular fibers [2]. Whether traps are ordered or disordered makes only a small difference in the rate at which a trap captures receptors provided the traps are dilutely distributed over the entire surface of the cell [19, 20].

Goldstein *et al.* [20, 21] dealt with two conflicting hypotheses about the coated pit recycling process. In the first, coated pits are recycled in the same site where its invagination occurred. In the second, coated pits are returned on random locations of the cell surface. In either case, the coated pit effectively has a finite lifetime. However they found that in general the results of Adam-Delbrück [1], and Berg-Purcell [5], obtained under the assumption that sinks have infinite lifetimes give good approximations for the dynamics of the experimental system analyzed: receptors for low density lipoproteins on human fibroblastic cells. This will also be true for more rapidly diffusing receptors. Most known cell surface receptors are in this category.

6. THE MODEL FOR A GENERAL RADIAL REINSERTION MODE

In this section we will extend the model presented in Wofsy, Echavarría and Goldstein [47]. The motivation for this generalization arises from the ideas presented by Keizer *et al.* [29] which claim that instead of the suggested uniform aggregation of receptors surrounding coated pits proposed by Robeneck and Hesz [38] a depleted concentration should be observed. A general model which gives the steady state radial concentration of receptors depending on an arbitrary insertion rate function will permit to evaluate the resulting steady state radial distribution function. This analysis is not possible if we only consider a step function form for the insertion mode. This was done by Wofsy, Echavarría and Goldstein [47] with the solely purpose of evaluating the potential of preferential LDL receptor reinsertion to enhance the trapping rate.

The model presented here will consider the case of a low density of coated pits on the cell surface. As it was already discussed this is the case for coated pits on human fibroblastic cells, at a normal temperature of 37°C. The assumption of an ordered system of stationary traps will be also, invoked. We will reduce the real multi-trap situation to its single trap approximation using the geometry introduced by Berg and Purcell, and associate to the trap a circular region of radius a centered on a disk of radius $b > a$.

The average radius of a coated pit on a human fibroblast is 10^{-5} cm (see Wofsy and Goldstein [48] for a review of the published measurements). The outer radius of the region we consider is chosen so that the area ratio $(\pi a^2)/(\pi b^2) = 0.01$. Thus, to model coated pits on human fibroblasts we take $a = 10^{-5}$ cm and $b = 10^{-4}$ cm.

In the context of the used approximation we will assume that the diffusing particles, *i.e.*, the receptors, are after synthesis inserted into the annulus about the trap, according to

a general radially symmetric insertion rate function $S(r)$. This will be identified with the number of receptors per unit area per unit time inserted at a distance r from the center of the trap, for $a \leq r \leq b$. In our study the mode of insertion proposed by Robeneck and Hesz [38], will be obtained as a particular characterization of the general radially dependent reinsertion rate function $S(r)$.

A steady state concentration density $C(r)$ of particles at a distance r from the center of the trap will be assumed to be maintained by a balance between the number of receptors trapped and the number of receptors inserted. This will necessarily set to zero the flux across the outer boundary of the annulus. Finally we assume that the only mechanism controlling the receptor movement is diffusion. Hence we have to consider the following steady state equation for the concentration of diffusing particles $C(r)$

$$D\nabla^2 C(r) + S(r) = 0, \qquad a \leq r \leq b. \tag{5}$$

The boundary conditions will be

$$C(a) = 0, \tag{6}$$

and

$$\int_{-\pi}^{\pi} \vec{J}\Big|_{r=b} \cdot \vec{n}\, d\theta = 0. \tag{7}$$

In Eq. (5) $S(r)$ represents the source term while boundary condition (6) is identified with the sink of radius a. In Eq. (7), $\vec{n}$ is the unitary normal vector pointing out radially from the origin towards (r, θ) and $\vec{J}$ represents the flux vector. The magnitude of $\vec{J}$ gives the net number of particles per unit time per unit length crossing a boundary; its units are particles/cm/s. Since we do not consider convection, just diffusion, then

$$\vec{J} = -D\nabla C. \tag{8}$$

That is, the net flux is proportional to the concentration gradient and in the opposite direction; particles tend to flow from areas of higher concentration to areas of lower concentration. Using the representation (8) and the polar coordinates form for $\nabla^2 C(r)$ and $\vec{n}$, we see that boundary condition (7) transforms into

$$\int_0^{2\pi} \left(-D \frac{\partial C}{\partial r}\Big|_{r=b} \right) d\theta = 0,$$

or equivalently, since C is a function of r alone, and $D > O$,

$$\frac{\partial C}{\partial r}\Big|_{r=b} = 0. \tag{9}$$

This implies that under the assumption of a radially and symmetric concentration $C(r)$, setting the net flux of particles across the boundary at $r = b$ to zero amounts to a reflecting boundary condition. Eqs. (5) to (7) define our model for the aggregation of receptors in coated pits in the presence of diffusion and a general radially dependent and symmetric mode of reinsertion for receptors.

7. DETERMINATION OF THE MEAN CAPTURE TIME τ_s AND APPLICATIONS

The solution $C(r)$ to Eq. (5) satisfying boundary conditions (6) and (9) is used in combination with Eq. (1) to find the mean capture time τ_s for particles diffusing in the annulus $\Omega = \{(r,\theta): a \leq r \leq b, 0 \leq \theta \leq 2\pi\}$, with initial positions determined by an insertion rate $S(r)$. As it is shown in the Appendix if $S(r)$ is an integrable function defined on $a \leq r \leq b$. The solution $C(r)$ to Eq. (5) satisfying boundary conditions (6) and (9) is for $D > 0$:

$$C(r) = \int_a^r \frac{\int_z^b uS(u)\,du}{Dz}\,dz. \tag{10}$$

Then, τ_s is found to be

$$\tau_s = \frac{\int_a^b \left(\frac{b^2}{2D}\ln\left(\frac{u}{a}\right) + \frac{a^2-u^2}{4D}\right) uS(u)\,du}{\int_a^b uS(u)\,du}. \tag{11}$$

As it is easily shown (see Appendix) when $S(r) = S$, S being a positive constant, we have $\tau_s = \tau$ for τ as given by Eq. (3). This indicates that when reinsertion of particles is uniform all over Ω, τ_s will give the result obtained by Berg and Purcell [5], using the mean capture time method described in Sect. 3. It can be shown (see Ref. [14]) that when $S(r)$ is continuous, monotonic in $[a,b]$, and differentiable in (a,b) then if $S(r)$ is increasing $\tau_s > \tau$ and if $S(r)$ is decreasing $\tau_s < \tau$.

As an example of the application of the model, $S(r)$ could be chosen in the form used by Wofsy *et al.* [47] to model the insertion mode envisioned by Robeneck and Hesz [38]. For this particular insertion mode receptor insertion is uniform on the region $\Omega_{ma} = \{(r,\theta): a \leq r \leq ma, 0 \leq \theta \leq 2\pi$, where $1 < m < b/a\}$. Correspondingly we have the insertion rate function,

$$S_m(r) = \begin{cases} S, & a \leq u < ma, \\ 0, & ma \leq u \leq b, \end{cases} \tag{12}$$

where S is a positive constant. In this case Eq. (11) gives

$$\tau_{S_m} = \frac{b^2m^2}{2D(m^2-1)}\ln(m) - \frac{2b^2 + (m^2-1)a^2}{8D}. \tag{13}$$

For τ given by Eq. (2) and for τ_{S_m} as given by the equation above, we have (see Ref. [14])

$$\tau_{S_m} < \tau. \tag{14}$$

This implies that in general the reinsertion mode proposed by Robenek and Hesz is a mechanism that reduces the mean capture time calculated on the assumption of diffusion and uniform reinsertion all over the annulus Ω.

Our model can be used to evaluate the suggested function of the plaques to shorten the time for LDL receptors to reach coated pits. For the values $a = 10^{-5}$ cm, $b = 10^{-4}$ cm

and $D = 4.5 \times 10^{-11}$ cm^2/sec which are respectively the values of the radius of a coated pit, the radius of the reference circle and the diffusion coefficient for human fibroblastic cells and LDL receptors, using Eq. (12). We conclude that halving τ requires a plaque radius $r_p = 3.2a$. This implies that unless insertion is restricted to a small portion of the cell surface ($\leq 10\%$), the capture rate does not increase dramatically (*i.e.*, it doubles at most) over the rate if receptors were inserted without restriction.

As another example of the application of our model, we estimate the effect on τ associated with a decreasing insertion mode of the form $S(r) = Sr^{-\alpha}$ where S is a positive constant, and $\alpha > 0$. From Eq. (11) we obtain (see Ref. [14]).

$$\tau_{S(\alpha)} = \begin{cases} \dfrac{1}{2D(b^{2-\alpha} - a^{2-\alpha})} \left[b^{4-\alpha} \ln\left(\dfrac{b}{a}\right) - \dfrac{b^{2-\alpha}(b^2 - a^2)}{2} \right. \\ \qquad \left. + \dfrac{b^{4-\alpha} - a^{4-\alpha}}{4-\alpha} - \dfrac{b^2(b^{2-\alpha} - a^{2-\alpha})}{2-\alpha} \right], & \text{for } \alpha \neq 2, 4; \\ \dfrac{b^2 \ln\left(\frac{b}{a}\right)}{2D} + \dfrac{a + a\ln\left(\frac{b}{a}\right) - b}{2D \ln\left(\frac{b}{a}\right)}, & \text{for } \alpha = 2; \\ \dfrac{1}{2D[b^{-2} - a^{-2}]} \left[\ln\left(\dfrac{b}{a}\right) - \dfrac{1 - \left(\frac{a}{b}\right)^2}{2} + \ln\left(\dfrac{b}{a}\right) - \dfrac{1 - \left(\frac{b}{a}\right)^2}{2-\alpha} \right], & \text{for } \alpha = 4. \end{cases} \tag{15}$$

Here we have used $\tau_s(\alpha)$ to denote the mean capture time given by Eq. (11) for an arbitrary value of the exponent which defines $S(r) = Sr^{-\alpha}$. For instance, for $\alpha = 4$ we calculated $\tau_{s(4)} = 0.935$ min. This corresponds to approximately one third of τ.

8. Discussion

Our model can contribute to the theoretical evaluation of the distribution pattern that must be observed when recycled receptors are inserted in plaques. In this vein we observe that, the solution $C(r)$ for the steady state concentration of LDL receptors around coated pits given by Eq. (10) increases for any particular characterization of $S(r)$, as a continuous and positive function [14]. Recalling that we obtained $C(r)$ under the additional assumption of a receptor transport controlled solely by diffusion, the increasing character of $C(r)$ precludes the possibility of a uniformly aggregated concentration of receptors around coated pits. Theoretically, the proposed plaques being an example of this effect are unobservable if diffusion is the fundamental controlling factor for the coated pit-receptor reaction step in receptor mediated endocytosis. In fact without loss of generality, lets consider and step like form for $S(r)$, as given by Eq. (12). Then Eq. (10) gives

$$C(r) = \begin{cases} \dfrac{I_{SR(ma)}}{\pi(m^2a^2 - a^2)} \left(\dfrac{m^2a^2}{2D} \ln\left(\dfrac{r}{a}\right) - \dfrac{(r^2 - a^2)}{4D} \right), & a \leq r \leq ma; \\ 0, & ma < r \leq b. \end{cases} \tag{16}$$

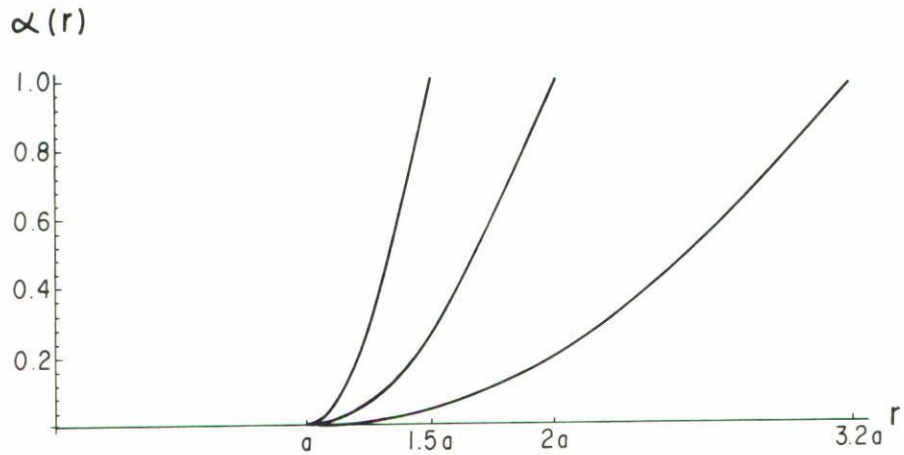

FIGURE 2. The behavior of the radial variation of the percentage $\alpha(r)$ of cell surface distributed LDL receptors. The recycled receptors were inserted in plaques of outer radius ma. We have considered the plots corresponding to $m = 1.5$, $m = 2$ and $m = 3.2$. For all plots $\alpha(ma) = 1$. The parameter a represents the radius of a coated pit. The radial distance r varies from a to ma.

The constant $I_{SR(ma)}$ stands for the total number of particles inserted in the plaque. We can observe that $C(r)$ as given by the above equation, varies form 0 to a maximum value $C(ma)$ and then drops to zero. This shows that receptors will not appear uniformly aggregated on the region $a \leq r \leq ma$. To illustrate our claim we can provide a plot of the radial variation of the proportion $\alpha(r)$ of diffusing particles within a plaque. To this aim, lets denote by $N(r)$ the number of particles distributed between the boundary of the coated pit and a circle of radius r. Then we must have

$$N(r) = \int_a^r 2\pi u C(u)\, du. \tag{17}$$

When in this integral $C(r)$ is given by Eq. (16), $N(ma)$ corresponds to the number of particles diffusing in a plaque of outer radius ma surrounding a coated pit. Then $\alpha(r)$ is given by the ratio $N(r)/N(ma)$. Figure 2 gives the behavior of $\alpha(r)$ for $a \leq r \leq ma$.

For a plaque of outer radius ma let us define its depletion annulus. This will be an annulus surrounding the coated pit, where 20% of the recycled receptors are inserted. Due to the concentration gradient imposed by $C(r)$ this annulus must be observable. For $m = 3.2$, we have a plaque that halves τ. The depletion annulus will be the region surrounding the coated pit with internal radius a and external radius $2a$. For $m = 1.5$ we have a plaque of outer radius which is close to the average radius of a coated pit. For this plaque 80% of the distributed receptor are found in an annular region of inner radius $1.18\,a$ and outer radius $1.5\,a$ that is, the depletion region, is an annulus with an area slightly smaller than 40% of the area of an average coated pit. The smaller the outer radius of the plaque the smaller its depletion annulus. Considering that the coated pit itself is a region for the aggregation of receptors, a plaque of outer radius $1.5\,a$ could be actually mistaken with the outermost portion of a coated pit. In virtue of this we may conclude that regions with an uniformly aggregated surface distribution of receptors

in the annuls $a \leq r \leq ma$ could be mistaken or unobservable under the assumption of receptor movement controlled solely by diffusion. If this is the case our model predicts that preferential reinsertion in plaques will lead to a depletion annulus which must be observable under suitable experimental exploration. Furthermore from a theoretical point of view, it is possible, to raise the conclusion that in case the plaques exist, as envisioned by Robeneck and Hesz necessarily the depletion annulus will be inexistent. That is, we will not detect a surface concentration gradient of distributed LDL receptors within a plaque. This independently of the size of their outer radii. This could be an evidence of a more active transport of LDL receptors to coated pits. If in fact diffusion is the controlling step for the aggregation of receptors in coated pits, the reported plaques might be fortuitous and more experimental evidence would be needed to elucidate this quarrel.

The aim of the experimental study conducted by Robeneck and Hesz [38] was to characterize further the human skin fibroblast membrane receptor for LDL. They reported that LDL particles bound to colloidal gold in junction with the surface replication technique can be used to visualize steps in the movement of their receptors in the plane of the lipid belayer. In their view, this experimental framework provided the first clear demonstration of the sequential clustering of LDL receptors near coated pits. They concluded that this effect is produced when recycled LDL receptors are inserted uniformly in annular regions surrounding coated pits. This contradicts the hypothesis of uniform receptor insertion all over the cell membrane reported by Goldstein *et al.* [22]. The reported plaques appear to cover a small fraction of the cell surface, as it would be required if they were to enhance LDL receptor capture substantially. Most plaques are comparable in size to coated pits with the mean plaque area corresponding to a plaque radius that is between one and two times the average coated pit radius. We have conjectured that for this radii plaques could be mistakenly identified. Furthermore, strictly speaking the existence of LDL-receptor plaques is still unproved. In the experiments described, the LDL-gold particles were highly multivalent and thus may have bound more efficiently to aggregated than single receptor [47]. If that is the case LDL gold particles could be actually found in coated pits producing an apparent non depleted concentration in the neighborhood of these structures. This reinforces our claim. In summary, the LDL-gold binding pattern may not reflect the true receptor distribution. The binding of LDL-gold may even alter the receptor or coated pit distribution as antibodies have done in other system [41, 34]. Aggregation of newly inserted LDL receptor in regions about coated pits is a controversial question.

Regarding the general question of the dependence of the refereed trapping rate on insertion modes, our model indicates (see Ref. [14]), that there could be infinitely many insertion rate functions $S(r)$ that will reduce the mean capture time τ by a fixed amount. Any insertion rate that decreases with distance r from the trap decreases the mean capture time τ. Nevertheless from a theoretical point of view an step like form for $S(r)$ as the mode of reinsertion proposed by Robeneck and Hesz will also in general reduce τ.

In the general situation the number $I_{S(r)}$ of particles inserted by $S(r)$ in the annular region $\Omega_r = \{(u, \theta) \mid a \leq u \leq r, 0 \leq \theta \leq 2\pi\}$ is given by the integral,

$$I_{S(r)} = \int_a^r 2\pi u S(u)\, du. \tag{18}$$

A reinsertion mode of the form $S(r) = Sr^{-\alpha}$ $(\alpha \geq 0)$ provides an example of a continuously decreasing rate function for which most receptors are recycled close to coated pits. This generates a mean capture time $\tau_{s(\alpha)}$ given by Eq. (15).

For this example the positive constant S is related to the total number of particles $I_{S(b)}$ inserted in Ω by means of the relationship [see Eq. (27)]

$$I_{S(b)} = \pi S \frac{(b^2 - a^2)}{a^2 b^2}.$$

As expected, we obtain $\tau_{s(0)} = \tau$. For $\alpha = 4$, $\tau_{s(4)}$ has a value of 0.935 min. This shows that an insertion rate function of the form $S(r) = Sr^{-4}$ can reduce the mean capture time τ to approximately one third of its value. We conjecture about other possible forms for the reinsertion mode. In particular, a continuously decreasing reinsertion which, as our example indicates could be an alternative to reinsertion in plaques to reduce τ.

A plaque whose outer radius is $2a$ will give a mean capture time of 0.78 min. This value is close to the figure calculated for $S(r) = Sr^{-4}$. On average the difference on capture time for particles inserted by this rate function and a plaque with outer radius $2a$ is 0.15 min.

The number of particles that a Robeneck and Hesz insertion rate function projects in the annulus Ω_{ma} has been already denoted by $I_{SR(ma)}$. The steady state assumption for the concentration of diffusing receptors implies $I_{SR(ma)}$ to be equal to $I_{S(b)}$. This independently of the choosing of $S(r)$. The number of particles inserted in the same region by the function $S(r) = Sr^{-4}$, is obtained replacing $r = ma$ and $S(r) = Sr^{-4}$ in Eq. (18). We get [see Eq. (28)]

$$I_{S(ma)} = \frac{I_s(b)b^2}{(b^2 - a^2)m^2}(m^2 - 1);$$

replacing in this equation the pertinent values for a and b, and considering the insertion mode characterized by a plaque of outer radius $2a$, we have $I_{S(2a)} = (0.76)I_{SR(2a)}$. That is, the rate function $S(r) = Sr^{-4}$ inserts in the plaque of outer radius $2a$ 76% of the total number of recycled receptors. If we consider that the mean capture time τ_s is comparable to τ_{S_m}, the particular insertion rate function $S(r) = Sr^{-4}$ seems to be a more efficient mechanism to reduce τ. For this insertion mode 24% of the recycled particles are inserted out of the plaque that gives a similar reduction on τ. A continuous and decreasing insertion rate function could be an alternate paradigm for the reduction of the mean capture time for LDL receptors by coated pits. If this is the case we can conjecture that within experimental limits the plaques reported by Robeneck and Hesz could be mistaken with the regions with the greater concentration of recycled receptors associated to a continuously decreasing insertion rate function. As it seems to be the case if preferential reinsertion must be very restricted to enhance the trapping rate, the topological difference of resulting surface aggregation patterns associated with an step and a continuous insertion function of the form used in the above example could be irrelevant.

The theoretical results we have presented allow us to asses the significance of any restriction of the insertion of LDL receptors (or any other receptors) to regions surrounding coated pits; *i.e.*, if we know the insertion rate $S(r)$ we can determine the mean time to

reach coated pits compared to the unrestricted case. As it is explained in Echavarría and Solana [15], if the binding of LDL receptors to coated pits is reversible a restricted insertion mode would be an efficient way to reduce trapping rates, although, in a more general setting more, experimental research is needed to identify other possible mechanisms which besides diffusion would explain the observed aggregation rates of LDL receptors in coated pits.

9. SUMMARY

The purpose of this study was twofold. On one hand we were interested in the theoretical evaluation of the claim raised by Robeneck and Hesz, that a non depleted concentration of LDL receptors must be observed in annular regions surrounding coated pits. We also addressed the problem of the determination of the mean capture time of LDL receptors by coated pits in the general case where their reinsertion mode is an arbitrary radially dependent and symmetric function.

One of the fundamental questions that theoretical studies on receptor mediated endocytosis have addressed concerns the role which diffusion plays on the control of the binding of receptors to coated pits. Using the mechanistic statistical theory of non equilibrium thermodynamics Keizer *et al.* [29] addressed this problem. For the same experimental system we are dealing in this paper they obtained the same lower bound for the binding rate constant k_+ reported by Goldstein *et al.* [20]. Furthermore assuming that reinsertion was uniform all over the cell surface Keizer and collaborators characterized the steady state concentration of unbound receptors around coated pits as a radially increasing function. Using the Berg Purcell approximation method, we raise here the same conclusion for a general insertion mode, and when diffusion is the controlling factor for the movement of LDL receptors. This can be easily verified using Eq. (20). We have also obtained the conclusion that under the fundamental control of diffusion for the movement of LDL receptors the plaques proposed by Robeneck and Hesz [38] are unobservable. If a plaque form aggregation pattern would be observed, we would need to invoke some other transport mechanism. In any event more experimental work must be performed to resolve this issue.

Regarding the effect of generalized preferential reinsertion in comparison with the Robeneck and Hesz assumption, we have exhibited a particular example of a continuously decreasing insertion rate function which seems to be relatively more efficient in reducing the mean capture time of LDL receptors by coated pits. In the general situation any continuous decreasing function will reduce the mean capture time of diffusing LDL receptors by coated pits.

ACKNOWLEDGMENTS

We are indebted with an anonymous reviewer for suggestions which improved our presentation. We also thank Diana Celina Rodríguez Davison for assistance in the preparation of the final manuscript.

APPENDIX

We first derive Eq. (10). To achieve this, replacing $\nabla^2 C(r)$ by $\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right)$ in Eq. (5) for $D > 0$ we obtain

$$\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) = -r\frac{S(r)}{D}.$$

Integrating with respect to r and applying boundary condition (9) we get

$$\frac{\partial C}{\partial r} = \frac{\int_r^b uS(u)\,du}{rD}. \tag{19}$$

Integration with respect to r gives

$$C(r) = \int_a^r \frac{\int_z^b uS(u)\,du}{Dz}\,dz, \tag{20}$$

for which we have $C(a) = 0$. This establishes Eq.(10).

From Eq. (19) it follows that

$$r^2\frac{\partial C}{\partial r} = \frac{r\int_r^b uS(u)\,du}{D}.$$

Integration by parts of the above equation for $a \le r \le b$ yields

$$r^2 C(r)\Big|_a^b - \int_a^b 2rC(r)\,dr = \int_a^b \frac{r\int_r^b uS(U)\,du}{D}\,dr.$$

From the preceding equation we obtain

$$\int_a^b 2\pi rC(r)\,dr = \pi b^2 C(b) - \int_a^b \pi\left[\frac{r}{D}\int_r^b uS(u)\,du\right]dr.$$

Finally using Eq. (20) to replace $b^2C(b)$ in the above equation will give

$$\int_a^b 2\pi rC(r)\,dr = \int_a^b \frac{\pi(b^2 - z^2)}{Dz}\left[\int_z^b uS(u)\,du\right]dz. \tag{21}$$

Denoting by $\langle C\rangle$ the average concentration of particles in the disk of radius b, we must have

$$\langle C\rangle = \frac{1}{\pi b^2}\int_0^b 2\pi rC(r)\,dr. \tag{22}$$

Denoting by k_{s+} the diffusion limited forward rate constant, depending on an arbitrary radially symmetric insertion rate $S(r)$ for $a \leq r \leq b$, then k_{s+} is defined by

$$k_{s+} = \frac{2\pi a D \left.\frac{\partial C}{\partial r}\right|_{r=a}}{\langle C \rangle},$$

i.e., k_{s+} is the flux of particles into the trap divided by the average particle concentration, or the number of particles hitting the trap per unit time, per diffusing particle.

Since we assumed a steady state concentration of particles, the number of particles trapped is the same as the number inserted into the annulus in a unit time. Consequently k_{s+} is also given by

$$k_{s+} = \frac{2\pi \int_a^b rS(r)\,dr}{\langle C \rangle}, \tag{23}$$

were $\langle C \rangle$ is defined by Eq. (22).

If we define τ_s to be the mean capture time for particles inserted in the annulus Ω according to a radially symmetric rate function $S(r)$ and moving by diffusion between an absorbing boundary at $r = a$ and a reflecting one at $r = b$, then by virtue of Eq. (1) we must have

$$\tau_s = \frac{\pi b^2}{k_{s+}}. \tag{24}$$

Using the results of Eq. (21) to replace $\langle C \rangle$ in Eq. (23), by virtue of Eq. (24) we get,

$$\tau_s = \int_a^b \left(\frac{b^2 - z^2}{2Dz}\right)\left[\frac{\int_z^b uS(u)\,du}{\int_a^b uS(u)\,du}\right] dz. \tag{25}$$

Changing the order of integration with respect to z we get

$$\int_a^b \left[\frac{b^2}{2D}\ln\left(\frac{u}{a}\right) + \frac{a^2 - u^2}{4D}\right] uS(u)\,du = \tau_s \int_a^b uS(u)\,du.$$

This establishes Eq. (11).

In the case when $S(r)$ is constant τ_s reduces to

$$\tau_s = \frac{\int_a^b \left(\frac{b^2}{2D}\ln\left(\frac{u}{a}\right) + \frac{a^2-u^2}{4D}\right) u\,du}{b^2 - a^2},$$

performing the involved integral we see that τ_s coincides with τ as given by Eq. (3).

Let $I_s(r)$ be the number of receptors inserted by the rate function $S(r)$ in the annular region

$$\Omega_r = \{(u, \theta) \mid a \leq u \leq r, 0 \leq \theta \leq 2\pi\},$$

then

$$I_s(r) = \int_a^r 2\pi u S(u)\, du. \tag{26}$$

Particularly $I_s(b)$ gives the number of receptors inserted by $S(r)$ in the reference annuls Ω.

For the particular choosing $S(r) = Sr^{-4}$ the constant S must satisfy

$$I_s(b) = 2\pi S \int_a^b u^{-3}\, du = \pi S \left(\frac{b^2 - a^2}{a^2 b^2} \right), \tag{27}$$

and correspondingly Eq. (26) gives

$$I_s(r) = \frac{I_s(b) a^2 b^2}{(b^2 - a^2)} \cdot \left[\frac{r^2 - a^2}{a^2 r^2} \right]. \tag{28}$$

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