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Time-dependent ligand current into a saturating cell performing chemoreception

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We determine the ligand current into a single spherical cell whose receptors become permanently blocked after binding ligands. Initially the cell is placed in a medium which contains ligands at uniform concentration. The analytical solution for the ligand distribution is obtained in terms of an integral over the solution at the cell surface. For the solution at the cell surface a nonlinear integral equation is derived which is solved numerically. We determine the time-dependent ligand current into the cell and the average number of free receptors in the cell surface as a function of time.

1. Introduction

Many processes in living cells are regulated by the flow of certain ligands into the cell. This information current depends on the details of the concentration gradient at the cell's surface. In the present paper we consider the ligand current into a single saturating cell. The cell is modeled as a sphere of radius R on which initially $N_0 \gg 1$ receptors for ligands are distributed; these receptors are assumed to be characterised by a single linear dimension s. In this model system, a receptor specific for the ligands in the exterior medium becomes permanently blocked after binding ligands. Thus, as more and more ligands are bound to the cell, fewer receptors remain free for further binding. This situation occurs in the binding of antibodies to liposomes which bear fluorescent haptens, as in the experiments of Petrossian and Owicki [1]. By measuring the fluorescence of a dilute system of these liposomes one obtains the average number of free receptors on the liposomes, as a function of time. We shall solve this time-dependent problem using the boundary condition approach of DeLisi and Wiegel [2,3].

We consider the problem in which a cell is placed at $t = 0$ in a medium with ligands at uniform concentration. The problem will be formulated mathematically in section 2. In section 3 an analytical expression for the solution is derived in terms of the solution on the cell surface. This leads to a nonlinear integral equation which is solved numerically as described in section 4. In section 5 the ligand current into the cell is shown for some representative parameters. We also calculate the time dependence of the average number of free receptors.

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2. Diffusion equation for the ligand distribution around a saturating cell

Consider a spherical cell of radius R, placed in a medium containing ligands at uniform concentration initially. In the membrane of the cell N_0 ($\gg 1$) receptors are immersed. These receptors

are assumed to be characterized by a single length s and can each capture only a finite number of ligands. The ligands in the exterior medium are assumed to follow diffusive paths and their translational motion is characterized by a diffusion coefficient D. The medium is macroscopically at rest. If $c(\boldsymbol{r},t)$ denotes the number density of ligands at position $\boldsymbol{r}$ and time t, the time evolution of $c(\boldsymbol{r},t)$ is governed by the diffusion equation [2]. In view of the spherical symmetry of the problem this reads

$$\frac{1}{D}\partial_t c(r,t) = \partial_{rr} c(r,t) + \frac{2}{r}\partial_r c(r,t) \tag{1}$$

where $r = |\boldsymbol{r}| > R$ and $t > 0$.

Initially, the cell is supposed to be in a homogeneous medium, in which the number density of ligands is constant

$$c(r,0) = c_0 \tag{2}$$

At the cell surface we impose a time-dependent boundary condition

$$c(R,t) - \alpha(t)\partial_r c(R,t) = 0 \tag{3}$$

This boundary condition expresses the fact that not every encounter of a ligand with the cell surface leads to binding of the ligand (cf. refs. 2 and 4). It is time dependent since the receptors are supposed to become blocked after binding ligands. In the course of time, more and more receptors will no longer be available for ligand capture and hence the probability that a ligand close to the membrane will be bound decreases. As shown in ref. 4, the function $\alpha(t)$ can be written in the form

$$\alpha(t) = \frac{\pi R^2}{sN(t)} \tag{4}$$

Here $N(t)$ denotes the average number of receptor sites at time t that are not blocked due to ligand binding. It will be clear that α is a strictly increasing function of time expressing the fact that in the course of time various receptors are blocked successively.

The average number of free receptors $N(t)$ can be expressed in terms of the ligand current into the cell. Let $J(t)$ be the total ligand current into the cell at time t, i.e.

$$J(t) = D\int_S\int \nabla c \cdot \boldsymbol{e}_r \, \mathrm{d}S = 4\pi R^2 D\partial_r c(R,t) \tag{5}$$

where S is the surface of the cell and $\boldsymbol{e}_r$ the outward pointing normal. The average number of free receptor sites, $N(t)$, is then determined by

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = -\kappa J(t) \tag{6}$$

with initial condition $N(0) = N_0$. Here, κ is a constant coupling the influx of ligands to the rate of change of the number of free receptors. If $\kappa = 0$ we again obtain the situation as treated in ref. 4. For $\kappa = 1$ a receptor can bind exactly one ligand. In general $0 \le \kappa \le 1$, and a receptor can bind a certain finite number of ligands before becoming blocked. A value for $\kappa \neq 1$ is related to situations in which a receptor has more than one binding site for ligands or when a cluster path of receptors is treated as one effective receptor. The total set of expressions, eqs. 1–6, determines the ligand distribution $c(r,t)$. Through the coupling of $N(t)$ with $\partial_r c(R,t)$ the boundary condition, eq. 3, is nonlinear in view of eq. 4.

The problem as formulated so far is quite complicated. It is, however, possible to relate it to the one-dimensional heat equation [5]. Indeed, letting

$$c(r,t) = c_0\left[1 - \frac{R}{r}f(r,t)\right] \tag{7}$$

and introducing the new variable $x = r - R$, one obtains for the function f

$$\frac{1}{D}\partial_t f(x,t) = \partial_{xx} f(x,t) \tag{8}$$

with initial condition

$$f(x,0) = 0 \tag{9}$$

and boundary condition

$$\partial_x f(0,t) - \left(\frac{1}{R} + \frac{1}{\alpha(t)}\right) f(0,t) = -\frac{1}{\alpha(t)} \tag{10}$$

By writing $c(r,t)$ in the form of eq. 7 one has transformed the problem to a one-dimensional problem for $f(x,t)$. This latter problem is much

easier to handle and can be treated analytically to a large extent.

Integrating eq. 6 and using eqs. 5 and 7 one obtains

$$N(t) = N(0) - 4\pi R^2 D\kappa c_0 \int_0^t \left[\frac{1}{R} f(0,\tau) - \partial_x f(0,\tau) \right] d\tau \tag{11}$$

which implies that eq. 10 can be written as

$$-\partial_x f(0,t) + hf(0,t) = h\varphi(t) \tag{12}$$

where

$$h = \frac{R + \alpha(0)}{\alpha(0) R} \tag{13}$$

and

$$\varphi(t) = \frac{1}{h\alpha(0)} + \frac{\beta(t)}{h}[1 - f(0,t)] \tag{14}$$

In this we represent $\alpha(t)$ as

$$\frac{1}{\alpha(t)} = \frac{1}{\alpha(0)} + \beta(t) \tag{15}$$

with

$$\frac{1}{\alpha(0)} = \frac{sN_0}{\pi R^2} \tag{16}$$

and

$$\beta(t) = 4sD\kappa c_0 \int_0^t \left[\partial_x f(0,\tau) - \frac{1}{R} f(0,\tau) \right] d\tau \tag{17}$$

The solution of eqs. 8–10 with $\alpha(t)$ as given in eq. 15 will be derived in section 3. It will be shown that $f(x,t)$ can be expressed as an integral over all previous times of the solution at the cell's surface.

3. Analytic solution for the ligand distribution

The determination of f proceeds in two steps. First, we find the integrating function

$$L(x,t) \equiv f(x,t) - \frac{1}{h} \partial_x f(x,t) \tag{18}$$

Second, we solve $f(x,t)$ from this equation.

It is straightforward to show that $L(x,t)$ satisfies the following system of equations

$$\partial_t L(x,t) = D\partial_{xx} L(x,t) \tag{19}$$

$$L(x,0) = 0 \tag{20}$$

$$L(0,t) = \varphi(t) \tag{21}$$

The solution to this problem can be found in ref. 5 and is given by

$$L(x,t) = \frac{2}{\sqrt{\pi}} \int_{x/2\sqrt{Dt}}^{\infty} \varphi\left(t - \frac{x^2}{4D\mu^2} \right) e^{-\mu^2} d\mu \tag{22}$$

Integrating eq. 18 formally, with this $L(x,t)$, one obtains [5]

$$f(x,t) = \frac{2h}{\sqrt{\pi}} \int_0^{\infty} e^{-h\eta} d\eta \times \int_{g(x,t,\eta)}^{\infty} \varphi(y(x,t,\eta,\mu)) e^{-\mu^2} d\mu \tag{23}$$

where

$$g(x,t,\eta) \equiv \frac{x + \eta}{2\sqrt{Dt}} \tag{24}$$

and

$$y(x,t,\eta,\mu) \equiv t - \frac{(x+\eta)^2}{4D\mu^2} \tag{25}$$

The function φ contains a constant part plus a time-dependent part. The integration in eq. 23 over this constant part can be performed explicitly. In total this yields

$$f(x,t) = \frac{R}{R + \alpha(0)} \left[1 - \operatorname{erf}\left(\frac{x}{2\sqrt{Dt}} \right) - e^{h(x+hDt)} \times \operatorname{erfc}\left(\frac{x}{2\sqrt{Dt}} + h\sqrt{Dt} \right) + \frac{2h}{\sqrt{\pi}} \int_0^{\infty} e^{-h\eta} d\eta \int_{g(x,t,\eta)}^{\infty} \alpha(0)\beta(y) \times (1 - f(0,y)) e^{-\mu^2} d\mu \right] \tag{26}$$

where erf and erfc are the error function and the complementary error function, respectively [6].

From this result, one observes that if the receptors can bind infinitely many ligands, i.e., the cell

does not saturate, the parameter κ in eq. 6 is zero, and the integral in eq. 26 is zero since $\beta = 0$. In this case eq. 26 reduces to the solution which was derived in ref. 4. Note also that the right-hand side of eq. 26 contains only the solution on the boundary $f(0,\tau)$, integrated over the entire history, since β can be expressed in terms of $f(0,\tau)$ only, as will be shown shortly. Finally, it should be pointed out that eq. 26 implies a nonlinear integral equation (by setting $x = 0$) from which $f(0,t)$ can be determined, thus closing the set of equations.

We will now express β in terms of $f(0,\tau)$ only and introduce a coordinate transformation for eq. 26 which expresses the integral equation for $f(0,t)$ in a form suitable for numerical treatment. In view of eq. 12 one may write

$$-\partial_x f(0,\tau) + \frac{1}{R} f(0,\tau) = \frac{1 - f(0,\tau)}{\alpha(0)} + \beta(\tau)[1 - f(0,\tau)] \tag{27}$$

Combination with eq. 17 gives

$$\beta(t) = 4D\kappa c_0 s \int_0^t \left[\frac{f(0,\tau) - 1}{\alpha(0)} + \beta(\tau)(f(0,\tau) - 1) \right] d\tau \tag{28}$$

which is equivalent to

$$\frac{d\beta(t)}{dt} = \frac{4D\kappa c_0 s}{\alpha(0)} [f(0,t) - 1] + 4D\kappa c_0 s \beta(t)[f(0,t) - 1] \tag{29}$$

However, this is an ordinary differential equation for $\beta(t)$, which has the solution

$$\alpha(0)\beta(t) = \exp\left[\int_0^t B(z)\, dz \right] \int_0^t B(z) \times \exp\left[- \int_0^z B(\vartheta)\, d\vartheta \right] dz \tag{30}$$

where

$$B(z) = 4D\kappa c_0 s [f(0,z) - 1] \tag{31}$$

Note that $\beta(t)$ is now expressed in terms of $f(0,\tau)$ only. Moreover, since $0 \le c(r,t) \le c_0$ for all r,t, one has $0 \le f(0,t) \le 1$ and hence B and β are negative. If we denote the solution for $\kappa = 0$ by $f_{\alpha(0)}(x,t)$, this implies $f(x,t) \le f_{\alpha(0)}(x,t)$ and hence

$$c(r,t) - c_{\alpha(0)}(r,t) \ge 0 \tag{32}$$

where $c_{\alpha(0)}$ is given by eq. 7 with f replaced by $f_{\alpha(0)}$. Hence, due to saturation of the cell, the ligand distribution in the exterior medium is higher than if the cell does not saturate.

Since solving $f(x,t)$ from eq. 26 analytically does not seem possible, we introduce a change of coordinates which renders eq. 26 more appropriate for numerical evaluation. Putting

$$\xi = h\eta \tag{33}$$

$$\nu = \mu - \frac{x + h^{-1}\xi}{2\sqrt{Dt}} \tag{34}$$

eq. 26 becomes

$$f(x,t) = \frac{R}{R + \alpha(0)} \left[1 - \operatorname{erf}\left[\frac{x}{2\sqrt{Dt}} \right] - e^{h(x + hDt)} \times \operatorname{erfc}\left[\frac{x}{2\sqrt{Dt}} + h\sqrt{Dt} \right] + \frac{2}{\sqrt{\pi}} \int_0^\infty e^{-\xi}\, d\xi \int_0^\infty \alpha(0)\beta(y_1) \times \left(1 - f(0,y_1) e^{-y_2} e^{-\nu^2}\, d\nu \right] \right] \tag{35}$$

where

$$y_1 = t \left[1 - \frac{(x + h^{-1}\xi)^2}{\left(2\sqrt{Dt}\,\nu + (x + h^{-1}\xi)\right)^2} \right] \tag{36}$$

$$y_2 = \frac{x + h^{-1}\xi}{\sqrt{Dt}} \left[\nu + \frac{x + h^{-1}\xi}{4\sqrt{Dt}} \right] \tag{37}$$

This expression is quite convenient for a numerical determination of $f(0,t)$, which forms the subject of section 4. Once $f(0,t)$ has been determined, all other quantities follow in a straightforward way.

4. Numerical determination of the solution at the cell surface

Setting $x = 0$ in eq. 35 one obtains a nonlinear integral equation for $f(0,t)$ which has the form

$$f = \frac{R}{R+\alpha(0)}\left(F + \frac{2}{\sqrt{\pi}} I(f)\right) \tag{38}$$

where $f \equiv f(0,t)$ and F is the solution at the cell's surface at constant $\alpha(0)$, i.e., $\kappa = 0$. This solution was derived in ref. 4 and is given by

$$F(t) = 1 - e^{h^2 Dt}\,\mathrm{erfc}(h\sqrt{Dt}) \tag{39}$$

The operator I is defined by

$$I(f)(t) = \int_0^\infty e^{-\xi}\,\mathrm{d}\xi \int_0^\infty \alpha(0)\beta(y_1) \times (1 - f(0,y_1)) e^{-y_2} e^{-\nu^2}\,\mathrm{d}\nu \tag{40}$$

where y_1 and y_2 are given by eqs. 36 and 37 upon setting $x = 0$. We may now define a sequence of functions (f_n) by

$$f_0 = \frac{R}{R+\alpha(0)} F \tag{41}$$

$$f_n = \frac{R}{R+\alpha(0)}\left(F + \frac{2}{\sqrt{\pi}} I(f_{n-1})\right) \tag{42}$$

with $n = 1, 2, 3, \ldots$. This sequence converges to $f(0,t)$ in the maximum norm that is

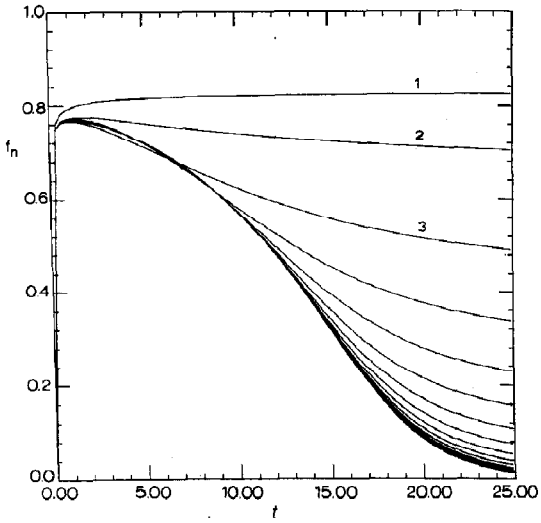
$$\lim_{n\to\infty} \max_{t>0} |f_n(t) - f_{n-1}(t)| = 0 \tag{43}$$

If $f(0,t)$ is known, $f(x,t)$ can be determined numerically through eq. 35. The integrations involved in eq. 40 were performed using Gauss-Laguerre integration [7]. The convergence of this scheme is relatively slow, but can be increased by defining the sequence

$$f_0 = \frac{R}{R+\alpha(0)} F \tag{44}$$

$$f_1 = \frac{R}{R+\alpha(0)}\left(F + \frac{2}{\sqrt{\pi}} I(F)\right) \tag{45}$$

$$f_n = \frac{R}{R+\alpha(0)}\left(F + \frac{2}{\sqrt{\pi}} I(2f_{n-1} - f_{n-2})\right) \tag{46}$$



a function of t which converge to the solution on the cell surface $f(0,t)$. In this calculation, $R = 5\times10^{-6}$ m; $\alpha(0) = 10^{-6}$ m; $D = 10^{-10}$ m^2 s^{-1} (values taken from ref. 2) and $\kappa c_0 s = 10^{-3}$ m^{-2}. A plot is shown of the first 15 iterands, according to the scheme defined by eqs. 44–46.

where $n = 2, 3, 4, \ldots$. After about 15 iterations this scheme has converged to $f(0,t)$ with an error of $O(10^{-3})$. In fig. 1 the sequence (f_n) is plotted for a characteristic set of parameters. The uppermost curve corresponds to the case $\alpha = \alpha_0$ which was treated analytically in ref. 4. As one observes from fig. 1, the time scale after which almost all receptors are blocked is about 25 s for these parameters.

In fig. 2, we have plotted $f(0,t)$ for various sets of parameters. In all cases we used $D = 1\times10^{-10}$ m^2 s^{-1}, $R = 5\times10^{-6}$ m (values taken from ref. 2) and $\alpha(0) = 1\times10^{-6}$ m (taken from ref. 4). One clearly recognizes that as κ decreases, it takes longer and longer before effectively all receptors are blocked due to binding of a ligand. If we define this time T_s as $f(0,T_s) = O(10^{-2})$, one notes from fig. 2 that $T_s \sim 1/(D\kappa c_0 s)$. In addition, it is clear that as κ decreases the curves $f(0,\xi)$ approach a common limit curve if one takes $\xi \sim \kappa t$. Since the ligand current into the cell and the average number of free receptors are directly related to $f(0,t)$, these quantities share this property as well.

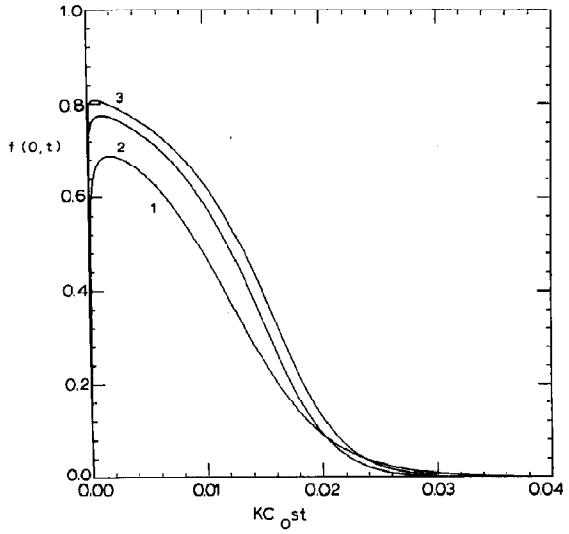


Fig. 2. The solution $f(0,t)$ on the cell surface with $R = 5\times10^{-6}$ m; $D = 10^{-10}$ m^2 s^{-1}; $\alpha(0) = 10^{-6}$ m and $\kappa c_0 s$ = (1) 10^{-2}, (2) 10^{-3} and (3) 10^{-4} m^{-2}.

In section 5, we turn to a discussion of the ligand distribution and the ligand current into the cell as well as to the time dependence of the average number of free receptors.

5. Ligand distribution and ligand current into the cell

Here, we first consider the ligand distribution and ligand current for some parameter sets. Next, the time dependence of the average number of free receptors on the cell surface is determined.

With the procedure described in section 4, the solution at the cell surface is determined for a representative choice of parameters. Using eq. 35 we then determined $f(x,t)$. A typical example of $f(x,t)$ is shown in fig. 3. An interesting feature is that in the long-time regime the solution near the cell surface is already quite close to the stationary value whereas at large distances (in units R) a significant deviation with respect to this stationary value exists and only slowly diffuses away. We also studied the corresponding ligand distribution $c(r,t)$. For short times the ligand distribution closely resembles the solution found in ref. 4 for $\alpha = \alpha(0) =$ constant. In the long-time regime, one again observes close to the cell surface a ligand concentration close to the limiting c_0, with small positive gradient, whereas far away (in units R) in the medium there is a relatively large deviation from the equilibrium state.

The ligand current into the cell is shown in fig. 4 for several sets of the parameters. For convenience we considered $J(t)/J_0$ where J_0 is the stationary ligand current into a perfectly absorbing sphere given by $J_0 = 4\pi RDc_0$ [2]. This can be written in terms of the solution on the cell surface using eqs. 5, 7 and 27 as

$$\frac{J(t)}{J_0} = \frac{R}{c_0}\partial_r c(R,t) = R\left[-\partial_x f(0,t) + \frac{f(0,t)}{R}\right]$$

$$= \frac{R}{\alpha(0)}(1-f(0,t))(1+\alpha(0)\beta(t)) \qquad (47)$$

For small times the ligand current closely resembles that found for the case of constant α ($\kappa = 0$). However, due to saturation, the ligand current decreases monotonically to zero as $t \to \infty$.

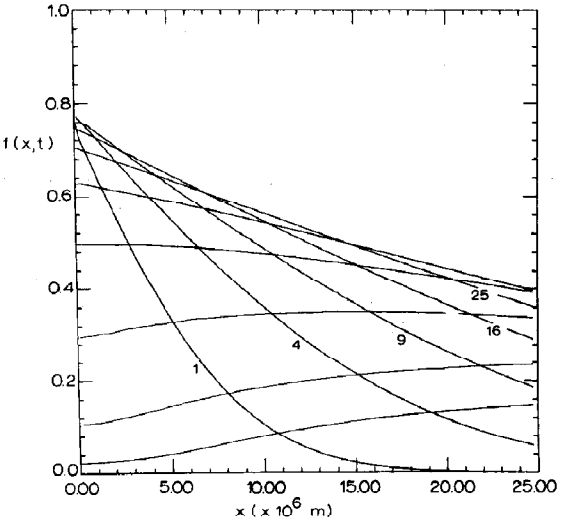


Fig. 3. The solution $f(x,t)$ as a function of x for various t values (Parameters as in fig. 1). The index on the curves corresponds to the time (in s) in units $\Delta t = 0.25$ s.

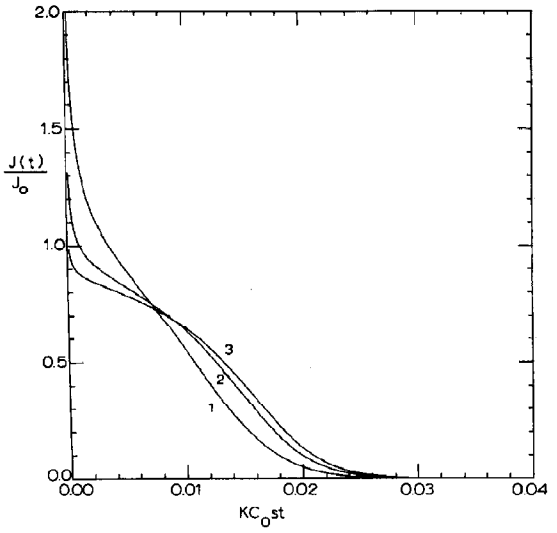


Fig. 4. The ligand current $J(t)/J_0$ as a function of time. Parameters as in fig. 2.

We now turn to the average number of free receptor sites as a function of time. As can be easily verified, one may write

$$\frac{N(t)}{N_0} = 1 + \alpha(0)\beta(t) \tag{48}$$

In fig. 5 we have plotted $N(t)/N_0$ as a function of time for a representative choice of parameters. The decrease in number of free receptors is quite sharp for short times. In this regime we have seen that the ligand current into the cell $J(t)$ is high (cf. fig. 4).

The number of free receptors is related to the fluorescence as a function of time of a system of liposomes bearing fluorescent receptors on its surface, as in the experiments of Petrossian and Owicki [1]. They placed the liposomes in a medium containing ligands which can bind to the fluorescent groups of certain receptors in the membrane of the liposome. After binding the fluorescent groups become extinct. Thus, the fluorescence decreases as more and more ligands are bound. In a dilute system of these liposomes the fluorescence as a function of time was measured [1]. Let $H(t)$ be the fluorescence at time t, then if we assume that the concentration of liposomes is so low that the ligand distribution around a cell is not influenced by the binding of ligands by other cells in the neighborhood, one expects that

$$\frac{H(t)}{H_0} = \frac{N(t)}{N_0} \tag{49}$$

Comparing the experimental results with the predictions of this model, one obtains good agreement in the short-time regime. In the long-time regime the predicted decrease of $N(t)$ it too sharp as compared to the experimental results. There are various possible effects, not included in the model, which could account for this discrepancy. In the long-time regime, the ligand distribution far away from the cell deviates significantly from the stationary value (cf. fig. 3 and its discussion). Hence, also in the dilute regime there is a coupling between the ligand currents of various cells. This may give rise to a fractional exponential decay in the long-time regime [8]. Another effect is related to an experimental problem. The fluorescent head groups can be located not only on the outside but also on the inside of the membrane. Perhaps in the long-time regime some of the free receptors from the inner membrane flip over to the outer membrane and are ready for ligand capture. Finally,

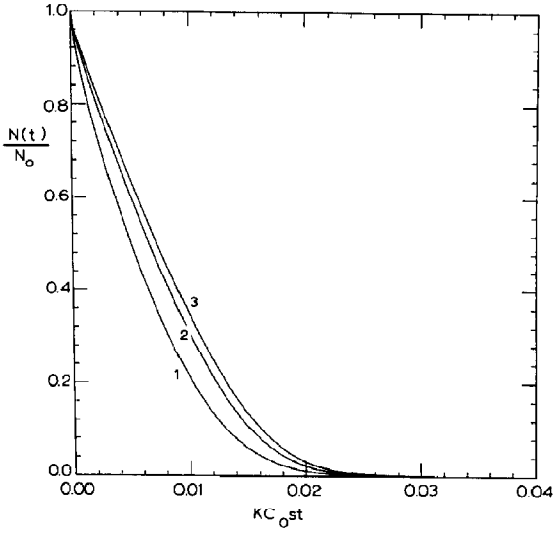


Fig. 5. The number of free receptors $N(t)/N_0$ as a function of time. Parameters as in fig. 2.

the reaction in which a ligand is bound to a receptor is reversible so that previously blocked receptors may release a ligand that is already bound and become fluorescent again. We hope to extend the model to include these effects.

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