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## APPENDIX A TO ARTICLE BPC 00907a

# DERIVATION OF RATE EQUATIONS FOR A THEORETICAL MODEL FOR THE PROPOSED KINETIC PATHWAY FOR REGULATION OF LDL RECEPTOR METABOLISM IN FIBROBLAST CELLS

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*Note: The references cited herein are listed on pages 195 and 196 of BPC 00907a*

On day 7, skin fibroblasts are incubated for 5 h at 37°C with varying concentrations of $^{125}$I-LDL. In the presence of LDL in the steady state, $k_{-m}$ will be much larger than $k_m$ and $k_{rd}$, while $k_2$ approaches zero. At a constant rate of synthesis, eq. 1 becomes eq. 2. The internalized receptor-LDL complex, $[R^mL]$, may be expressed as

$$\frac{d[R^mL]}{dt} + (k_{-3} + k_d)[R^mL] = k_L[R^sL],$$

multiplying both sides by $e^{(k_{-3}+k_d)t}$

$$\int_0^t d[R^mL]e^{(k_{-3}+k_d)t} = \int_0^t k_L[R^sL]e^{(k_{-3}+k_d)t}dt \quad \text{(A1)}$$

With a proper substitution, eq. A1 becomes

$$[R^mL] = \frac{k_L}{(k_{-3}+k_d)}\left\{[R^sL] - [R^sL]_0 e^{-(k_{-3}+k_d)t}\right\} + [R^mL]_0 e^{-(k_{-3}+k_d)t} \quad \text{(A2)}$$

On day 7 at initial time, $t = 0$, $[R^mL] = 0$, and $[R^sL]_0 = 0$. After 3 h of incubation with $^{125}$I-LDL at 37°C, the system establishes a dynamic equilibrium. The amount of $^{125}$I-LDL bound to the receptor, $[R^sL]$, and the amount of $^{125}$I-LDL contained within the cell, $[R^mL]$, remain relatively constant. Thus, the equilibrium relationship between $[R^mL]$ and $[R^sL]$ under the steady-state conditions may be expressed as

$$[R^mL] = \frac{k_L[R^sL]}{(k_{-3}+k_d)}$$

where $[R^sL] = \frac{k_2[L]}{k_L}[R^s]$ and $[R^s] = \frac{k_1[A]}{a}$ (A3)

Cell surface binding, internalization, and degradation of $^{125}$I-LDL at 37°C as a function of $^{125}$I-LDL concentration may be expressed as follows:

$$\frac{d}{dt}[R^sL]e^{k_L t} = k_2[R^s][L]e^{k_L t} \text{ which yields} \quad \text{(A4)}$$

$$[R^sL] - [R^sL]_0 e^{-k_L t} = \frac{k_2[L]}{k_L}\left\{[R^s] - [R^s]_0 e^{-k_L t}\right\} \quad \text{(A5)}$$

at $t = 0$, $[R^sL] = 0$ and $[R^s]_0 = [R^s]$, in which eq. A5 becomes

$$[R^sL] = \frac{k_2[L]}{k_L}\left\{[R^s] - [R^s]_0 e^{-k_L t}\right\} \quad \text{(A6)}$$

Substitution of eq. A6 into eq. A4 under steady-state conditions yields

$$[R^mL] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{[R^s] - [R^s]_0 e^{-k_L t}\right\} \quad \text{(A7)}$$

Cell surface receptor concentration, $[R^s]$, may be evaluated from eq. 2.

$$\frac{d[R^s]}{dt} = k_1[A] - k_2[R^s][L] + k_{-3}[R^mL]$$

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Substituting eq. A7 into this expression, it is possible to solve for the linear differential equation,

$$\frac{d[R^s]}{dt} + \left(\frac{k_2[L]k_d}{k_{-3}+k_d}\right)[R^s] = k_1[A] - \left(\frac{k_2[L]k_{-3}}{k_{-3}+k_d}\right)[R^s]e^{-k_L t} \quad (A8)$$

Multiply both sides by $e^{at}$, where $a = [k_2[L]k_d/(k_{-3}+k_d)]$. Then solve for $[R^s]$, the surface receptor concentration.

$$\int_0^t de^{at}[R^s] = \int_0^t \left\{k_1[A]e^{at} - \frac{k_2k_{-3}[L]}{k_{-3}+k_d}[R^s]_0 e^{(a-k_L)t}\right\}dt \quad (A9)$$

$$[R^s] = \frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]k_{-3}}{(k_{-3}+k_d)(a-k_L)} \times [R^s]_0(e^{-k_L t} - e^{-at}) + [R^s]_0 e^{-at}$$

Eq. A9 may be used to evaluate surface receptor concentration (eq. 3).

The concentration of cell surface receptor-LDL complex, $[R^sL]$, may be evaluated from eq. A6 with a proper substitution of eq. A9 (eq. 4).

$$[R^sL] = \frac{k_2[L]}{k_L}\{[R^s] - [R^s]_0 e^{-k_L t}\}$$

$$= \frac{k_2[L]}{k_L}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]k_{-3}}{(k_{-3}+k_d)(a-k_L)}[R^s]_0(e^{-k_L t} - e^{-at}) - [R^s]_0(e^{-k_L t} - e^{-at})\right\}$$

$$[R^sL] = \frac{k_2[L]}{k_L}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]-k_L}{a-k_L}[R^s]_0(e^{-k_L t} - e^{-at})\right\} \quad (A10)$$

The concentration of internalized $[R^mL]$, may be expressed as

$$[R^mL] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]k_{-3}}{(k_{-3}+k_d)(a-k_L)}[R^s]_0(e^{-k_L t} - e^{-at}) + [R^s]_0 e^{-at} - [R^s]_0 e^{-k_L t}\right\}$$

noting that $[R^s]$ from eq. A9 was substituted into eq. A7. Thus,

$$[R^mL] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]-k_L}{a-k_L}[R^s]_0(e^{-k_L t} - e^{-at})\right\} \quad (A11)$$

Under steady-state conditions,

$$[D] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]-k_L}{a-k_L}[R^s]_0(e^{-k_L t} - e^{-at})\right\}k_d^{LDL}t \quad (A12)$$

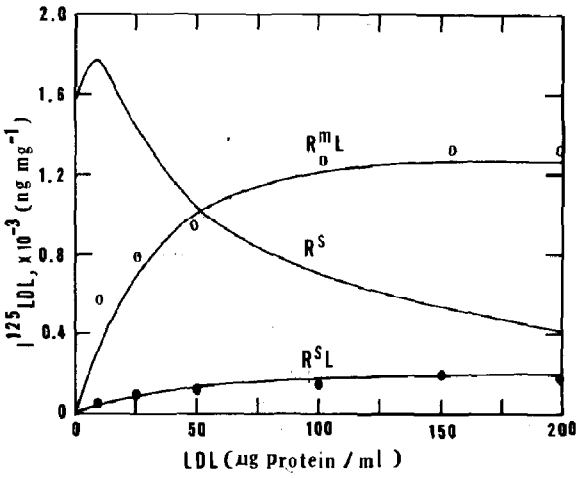
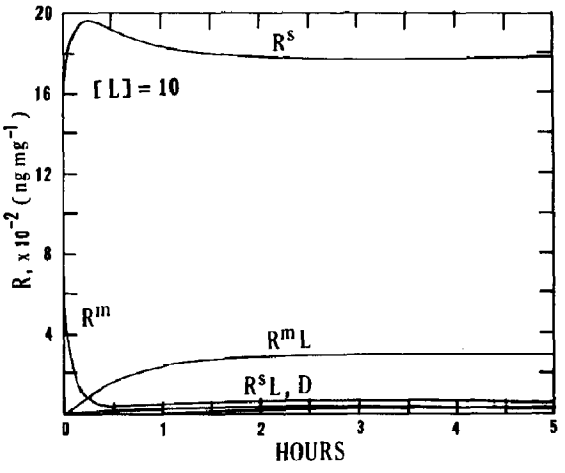



Fig. 6. Cell surface receptor binding and distribution of endocytotic species in a steady state in human fibroblasts as a function of $^{125}$I-LDL concentration. (○) Heparin-resistant fraction, (●) surface receptor-bound LDL; these experimental data were taken from fig. 1A and B of ref. 37. These plots were generated based on five differential equations in which the concentrations $[R^mL]$, $[R^sL]$, and $[R^s]$ were solved using the Runge-Kutta approximation. The best known kinetic parameters were used to simulate eq. 1.

while under non-steady state conditions,

$$[D] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]-k_L}{a-k_L}[R^s]_0(e^{-k_L t}-e^{-at})\right\} \times (1-e^{-k_d^{LDL}t}) \quad \text{(A13)}$$

Eqs. A9–A11 (eqs. 3–5) were used to evaluate the endocytotic species distributions as a function of time. Appropriate statistical tests were performed to evaluate the global goodness of fit of the $R^2$ value of each data point taken from Brown and Goldstein's experiment (fig. 1A and B of refs. 35 and 37).




It is now possible to evaluate all of the rate constants using eqs. A10 and A11, using the data reported by Brown and Goldstein in the steady state shown in tabular form below, within error limits of ±10%. A unit of $^{125}$I-LDL binding activity is expressed as that activity that will bind 1 ng $^{125}$I-LDL per mg cell protein after 5 h of incubation. 1 μg/ml LDL = 0.33 nmol. 1 μg protein/ml LDL = 1.414 nM LDL = 42.86 (μg/ml) LDL.

| LDL (μg protein/ml) | LDL (nM) | $R^sL$ (ng/mg) | $R^mL$ (ng/mg) |
|---|---|---|---|
| 10 | 14.14 | 68 | 590 |
| 25 | 36.36 | 96 | 850 |
| 50 | 70.71 | 125 | 930 |
| 100 | 141.43 | 164 | 1250 |
| 150 | 212.14 | 185 | 1350 |
| 200 | 282.86 | 192 | 1380 |

This simulation is based on two papers by Brown and Goldstein, one of which reported results (fig. 1A and B of ref. 37) in terms of μg protein per ml LDL and the second (fig. 1 of ref. 35) in terms of μg per ml LDL, although the results are identical. Our simulation considered

Fig. 7. The kinetic plots shown here and in figs. 8–10 were generated by the numerical solution of five differential equations of eq. 1, using the Runge-Kutta approximation method based on the kinetic parameters evaluated from eqs. 3–5. $k_2 = 0.025$ and [LDL] concentration varied from 10 to 200 μg protein/ml. $k_L = 24.0$, $k_d^{LDL} = 0.005$.

| | $k_1[A]$ | $R^m$ | $R^s$ | $R^sL$ | $R^mL$ | $D$ |
|---|---|---|---|---|---|---|
| $\frac{d[R^m]}{dt}$ | (6.0) | $-(k_{-m}+k_{rd})$ $-(10.05)$ | $k_m$ (0.1) | 0 | $k_{-3}$ (1.50) | 0 |
| $\frac{d[R^s]}{dt}$ | 0 | $k_m$ (10.0) | $-(k_m+k_2[L])$ $-(0.35 \rightarrow 5.1)$ | 0 | 0 | 0 |
| $\frac{d[R^sL]}{dt}$ | 0 | 0 | $k_2[L]$ $(0.25 \rightarrow 5.0)$ | $-k_L$ $-(24.0)$ | 0 | 0 |
| $\frac{d[R^mL]}{dt}$ | 0 | 0 | 0 | $k_L$ (24.0) | $-(k_{-3}+k_d)$ $-(1.505)$ | 0 |
| $\frac{d[D]}{dt}$ | 0 | $k_{rd}$ (0.05) | 0 | 0 | $k_d^{LDL}$ (0.005) | 0 |

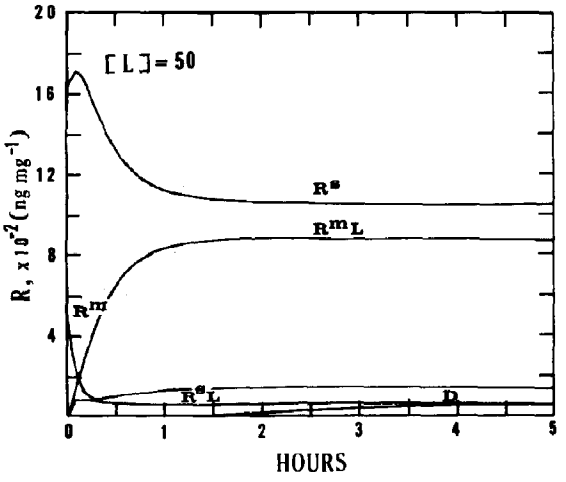



Fig. 8. See legend to fig. 7.

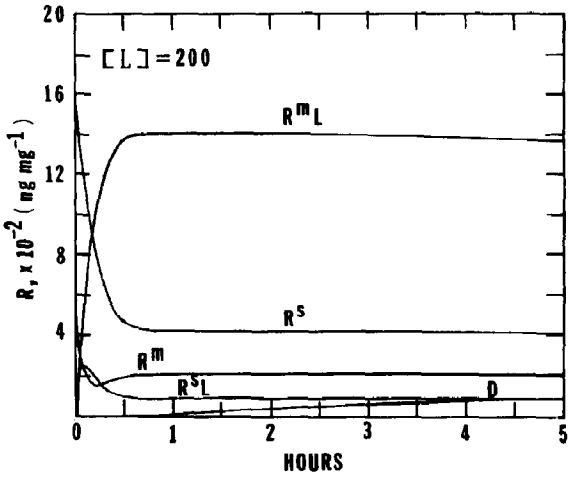



Fig. 10. See legend to fig. 7.

both types of units. The simulation of cell surface receptor binding [$R^sL$], internalization [$R^mL$], and concentration of surface receptor [$R^s$] in terms of $\mu$g protein per ml LDL are shown in fig. 6. In this instance, the best known kinetic parameters were used to simulate five differential equations which were then solved using the Runge-Kutta approximation method. These simulated curves are in quite good agreement with experimentally obtained values [37], which were apparently measured when the system was in a steady state at the end of a 5 h period.

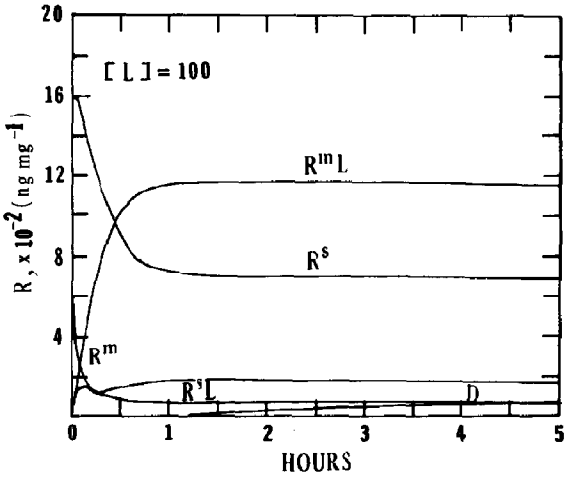



Fig. 9. See legend to fig. 7.

No matter which unitary measure was used in the simulation, the resultant data suggest the identical conclusion – that the degradation of LDL and the build-up of cholesterol apparently do not affect the down-regulation of receptor synthesis.

Figs. 7–10 show the macromolecular distribution of the endocytotic species of the LDL-receptor complex as a function of time, for several fixed concentrations of LDL. Values for $R^s$, $R^mL$, and $R^sL$ at the steady state were used in simulating fig. 6, and gave a good fit to Brown and Goldstein's data [37]. The macroscopic endocytotic species distribution reaches a steady-state level within 1 h in every case, although LDL concentration varied from 10 to 200 $\mu$g protein/ml. The hump which appears at concentrations up to 75 $\mu$g protein/ml gradually disappears at higher protein concentrations, and is probably due to the translocation of receptors from intracellular compartments to the cell surface and to the effects of washing. Under steady-state conditions, after the cells have been incubated in the presence of LDL for 5 h, the time required for a receptor to traverse the entire endocytotic pathway is about 60 min.