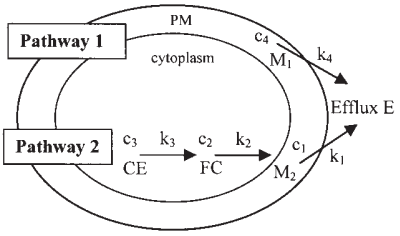

FIGURE 1: Schematic representation of proposed mathematical model. The rate constants, $k_1 - k_4$, are effective rate constants. The cholesterol domains are labeled $c_1 - c_4$ and have been associated with M_2, FC, CE, and M_1, respectively, based on previous observations (*16*). M_1 and M_2 are indicating two different plasma membrane domains. FC and CE stands for free cholesterol and cholesterol esters in the cytoplasm.

cellular compartments (e.g., plasma membrane cholesterol and cytosol). The concentrations in these models are expressed in percentages of the total cholesterol in the system and the rate constants in unit time (h). The differential equations from the proposed model as schematically shown in Figure 1 are as follows:

$$\frac{dE}{dt} = k_1 c_1(t) + k_4 c_4(t)$$

with

$$c_3(t) = c_3^0 e^{-k_3 t} \text{ and } c_4(t) = c_4^0 e^{-k_4 t}$$

where c_3^0 and c_4^0 are the respective cholesterol concentrations at time zero and

$$\frac{dc_1(t)}{dt} = -k_1 c_1(t) + k_2 c_2(t)$$

$$\frac{dc_2(t)}{dt} = k_3 c_3(t) - k_2 c_2(t)$$

This set of first-order linear equations can be integrated and substituted to obtain an expression of E (eq 2):

$$E = \frac{-k_1 k_2 c_3^0}{(k_2 - k_3)(k_1 - k_3)} \exp(-k_3 t) - \frac{A_1 k_1}{(k_1 - k_2)} \exp(-k_2 t) - A_2 \exp(-k_1 t) - c_4^0 \exp(-k_4 t) + A_3$$

A_1, A_2, and A_3 are integration constants. The boundary conditions are such that the efflux $E = 0$ at time zero, which allows the determination of A_3. c_2, c_1, and c_3 are greater than zero for all t which limits the choice of A_1 and A_2.

If reverse rate constants are incorporated into the model there is no longer an analytical solution. To solve such a model a Runge−Kutta algorithm was used to obtain an expression for E. The calculated values for the efflux E over time was then fitted to the experimental data using a nonlinear least-squares curve fitting program (Microsoft Excel 2000). The fitting of the theoretical expression to the data was evaluated using normalized squares of the residuals as an error function, ERF:

$$\text{ERF} = \frac{1}{\sum_i y_{obs}^2(i)} \times \sum_i [y_{obs}(i) - y_{calc}(i)]^2 \qquad (3)$$

where y_{obs} and y_{calc} are the experimental and theoretical values for the cholesterol efflux, respectively. Although each data point counts as equal weight in this error function, differences between data and theory for greater y's will dominate the error function; this error function is therefore suitable (and widely used) where the systematic error for small values is larger than for greater values. A fit is regarded as unsuitable for the experimental data when 1 > ERF > 0.1, reasonable when 0.1 > ERF > 0.01 and excellent when 0.01 > ERF > 0.001.

While the error function describes the overall quality of the fit it does not provide any statistical evaluation of the established regression coefficient (in this case the rate constants). The standard deviation of the regression coefficient k_i is given by (*24*)

$$\sigma_i = \sqrt{P_{ii}^{-1}}\, \text{SE(y)} \qquad (4)$$

where $\mathbf{P}_{ii}^{-1}$ is the ith diagonal element of the inverse of the $\mathbf{P}_{ij}$ matrix,

$$P_{ij} = \sum_{n=1}^{N} \frac{\delta E_n}{\delta k_i} \frac{\delta E_n}{\delta k_j}$$

with $\delta E_n/\delta k_i$ the partial derivative of the efflux function with respect to k_i evaluated at the time point t_n and

$$\text{SE(y)} = \sqrt{\frac{\sum (y_{obs} - y_{calc})^2}{N - K}}$$

and N the number of data points and K the number of regression coefficients to be determined. The differential terms $\delta E_n/\delta k_i$ can be calculated for each data point by numerical differentiation. The term k_i is varied by a small amount from its optimized value while the others k_j terms are held constant. This process is repeated for each of the K regression factors, then the cross product $(\delta E_n/\delta k_i)(\delta E_n/\delta k_j)$ for each of the N data points is obtained and the sum $\sum(\delta E_n/\delta k_i)(\delta E_n/\delta k_j)$ is computed. That allows the construction of the terms of the $\mathbf{P}_{ij}$ matrix which is then inverted and the terms along the main diagonal of the inverse matrix are then used to calculate the standard deviations of the coefficients using eq 4.

RESULTS

Sterol Loading of and Efflux from THP-1 Macrophages. As previously shown, model foam cells can be created in vitro by exposing mature macrophages to acetylated LDL (AcLDL) for 48 h which leads to an increased cholesterol content (~92 nmol sterol/mg of cell protein versus ~21 nmol/mg for nonloaded cells) of which between 30 and 60% (mean 46%) is esterified (see Table 1). By changing the ratio of 7KAcLDL to AcLDL in the loading media from 0:1 to 1:3 macrophages can be both cholesterol loaded and selectively and progressively enriched with 7K. The percentage of the 7K in terms of total sterol content can be increased from 0 to 20% without inducing cell death, as measured by lactate dehydrogenase release and cell protein content.

Uptake of lipoprotein enriched with ^{3}H-labeled cholesterol allows rapid analysis of subsequent cholesterol efflux. Efflux to apoA-1 (25 μg/mL) was started after an overnight