Determination of the optimal priming dose for achieving an isotopic steady state in a two-pool system: application to the study of cholesterol metabolism

D. B. ZILVERSMIT* and RICHARD A. WENTWORTH

Graduate School of Nutrition and Section of Biochemistry and Molecular Biology, Division of Biological Sciences and the Department of Physical Biology, Cornell University, Ithaca, New York 14850

ABSTRACT The daily administration of labeled cholesterol to humans or animals leads to an isotopic steady state. The specific activity of plasma cholesterol in the isotopic steady state gives information about the fraction of plasma cholesterol derived from endogenous and exogenous sources. A method, based on a two-pool model, is presented which allows the estimation of an optimal priming dose of labeled cholesterol whereby the time to reach the isotopic steady state is reduced to a minimum. A graphic procedure is presented which allows the estimation of an optimal priming dose for two-compartment systems with widely differing characteristics.

SUPPLEMENTARY KEY WORDS isotope kinetics

Recent studies on the metabolism of cholesterol in man have utilized the two-compartment model to calculate turnover rates and pool sizes (1, 2). One of the compartments is assumed to include plasma and all tissues in rapid isotopic equilibrium with plasma, whereas the second one is assumed to exchange cholesterol with the first compartment at a relatively slow rate. These investigators of cholesterol turnover in man employed the single injection technique, whereas others have used to advantage daily dosage of labeled cholesterol until an isotopic steady state is achieved (3–5). In this last method one may calculate the fraction of plasma cholesterol which is derived from synthesis and from the diet without assuming a particular model for the cholesterol metabolizing pools. The following two disadvantages are present in the repeated dosage method: (*a*) the time required to approach the isotopic steady state may be extraordinarily long, and (*b*) if additional parameters of cholesterol turnover are desired, one must stipulate a model or have independent information about cholesterol absorption or sterol excretion.

Grundy and Ahrens (5) have combined the single and continuous dosage techniques for a study of cholesterol metabolism in man. They aimed to minimize the time for isotopic equilibration, but they did not attempt to find the best combination of dosages to accomplish this. If preliminary data, from the literature or from pilot experiments, are available, one can choose the relative size of the daily dose and the single (priming) dose so as to minimize the time for reaching isotopic equilibrium as well as the total dose of isotope administered to a given patient.

METHOD

According to Goodman and Noble (1) the kinetics of cholesterol metabolism in man can be described by a two-compartment model. One of the compartments (A) consists of tissues in rapid equilibrium with plasma cholesterol, whereas the cholesterol in the other compartment (B) is in relatively slow equilibrium with that of compartment A. When labeled cholesterol is introduced continuously into compartment A, the amount of label in that compartment follows a time course:

* Career Investigator of the American Heart Association.

$$X^* = \frac{C_1}{\alpha}(1 - e^{-\alpha t}) + \frac{C_2}{\beta}(1 - e^{-\beta t}) \qquad \text{eq. (1)}$$

in which X^* is the amount of labeled cholesterol in compartment A (dpm) at time t, and C_1, C_2, α, and β are constants.[1] The dose rate of label, that is dpm administered per unit time is $\left(\frac{dX^*}{dt}\right)_{t=o}$, which equals $(C_1 + C_2)$.

In the experiments of Grundy and Ahrens (5), a daily oral dose of radioactive cholesterol was employed. Although such a dosing schedule only approximates the continuous administration of label described by eq. (1), the approximations should be quite good because cholesterol turnover is a relatively slow process. In subsequent discussions the dose of radioactive cholesterol administered "continuously" will be $(C_1 + C_2)$ dpm/day. By differentiation of eq. (1), it is possible to calculate the time course of the amount of labeled cholesterol in compartment A after administration of a single dose (Y_0^*). If the single dose is expressed as a multiple (M) of the daily dosage rate $(C_1 + C_2)$, the single "priming" dose is:

$$Y_0^* = M(C_1 + C_2) \qquad \text{eq. (2)}$$

The amount of labeled cholesterol in compartment A after such a priming dose will be:

$$Y^* = M(C_1 e^{-\alpha t} + C_2 e^{-\beta t}) \qquad \text{eq. (3)}^2$$

The question to be answered is: given certain values for C_1, C_2, α, and β, what is the optimal combination of single (priming) dose and repeated doses to assure the minimal time for reaching a constant specific activity in plasma? Mathematically the question is not yet in the proper form. A constant isotope concentration in plasma is reached only at infinite time. Therefore, we must be satisfied to determine the dosage schedule which will achieve, at the earliest time, a concentration of label in plasma within a certain percentage of the steady-state value. In the subsequent discussion we shall consider the system to be in the isotopic steady state when the plasma isotope concentration remains within 5 (or 1)% of the theoretical steady-state value.

When a subject is given a combined dose schedule such that the priming dose is M times the daily dose, the amount of labeled cholesterol in compartment A will be:[3]

$$G^* = X^* + Y^* \qquad \text{eq. (4)}$$

In Fig. 1 are plotted the values for G^* against time (days) for six different values of M. When M is zero, the curve describes the well-known situation of repeated constant dosage. By inspection it is evident that the isotopic steady state is reached fastest when M is about 40 (third curve from the top). The optimum value for M may readily be estimated by a computer.

The curves in Fig. 1 represent computer outputs for values of C_1/C_2, α, and β which are reasonably close to those calculated for a two-pool cholesterol model by Goodman and Noble (1). It is evident that for values of M equal to 10 or 20, a definite minimum in the curves exist, but that for $M = 40$, the minimum (even though it exists) is hardly visible. For values of M larger than 50, no minimum is present. The time at which a minimum occurs is calculated from $dG^*/dt = 0$ which is solved to yield:

$$t_{\min} = \frac{1}{(\alpha - \beta)} \ln \frac{(\alpha M - 1)C_1}{(1 - \beta M)C_2} \qquad \text{eq. (5)}$$

Because the function under the log sign must be positive, a solution for this equation exists only for values of M between $1/\alpha$ and $1/\beta$. For the example in Fig. 1, the curves exhibit a minimum for values of M between 6.67 and 50. Only for values of M between 8.06 and 50 do these minima occur at positive values of t. Within these limits of M, the amount of isotope in plasma decreases from zero time, when the priming dose is given, until a minimum is reached and then gradually increases toward the steady-state value. As may be seen in Table 1, for relatively low values of M, the times required to reach an isotopic steady state ($\pm 5\%$) are still relatively long. At a priming dose 20 times the daily dose a minimum plasma isotope concentration is found 20 days after the beginning of the dosage schedule, but it takes a total of 102 days to reach the isotopic steady state. At a priming dose of 40 times the daily dose, the curve starts well above

[1] Ordinarily the constants C_1, C_2, α, and β are determined from a semilogarithmic plot of radioactive cholesterol (dpm) in compartment A vs. time after giving a single dose of labeled cholesterol. This curve is easily decomposed into two straight lines. C_1 and C_2 are the intercepts of these lines with the Y axis, while α and β are rate constants calculated from the half times ($t_{1/2}$) of the two exponentials, for example, $\beta = \ln 2/t_{1/2} = 0.693/t_{1/2}$, where $t_{1/2}$ is the longer of the two half times. The constants can also be obtained by a similar procedure when isotope is administered at a constant rate.

Because one normally determines the *specific activities* of plasma cholesterol instead of the *total amount of label* in compartment A, it should be noted that identical values for α and β are obtained from the specific activity–time curve as from a radioactivity–time curve. Numeric values of the intercepts obtained from the two types of curves will differ by a constant factor. However, the ratio of the intercept values will be the same, and it is only the ratio of C_1/C_2 in addition to α and β that are needed for the subsequent calculations (see Fig. 3).

[2] M has the dimension of time as shown by eq. (2):

$$M = Y_0^*/(C_1 + C_2) = \frac{\text{dpm}}{\text{dpm/day}}$$

[3] Labeled cholesterol in plasma is, per definition, in rapid equilibrium with that of other tissues in compartment A. It is, therefore, permissible to use plasma cholesterol radioactivities per ml or plasma cholesterol specific activities instead of G^*. The curves will differ from G^* by a constant factor, which will not affect the interpretation of the results.

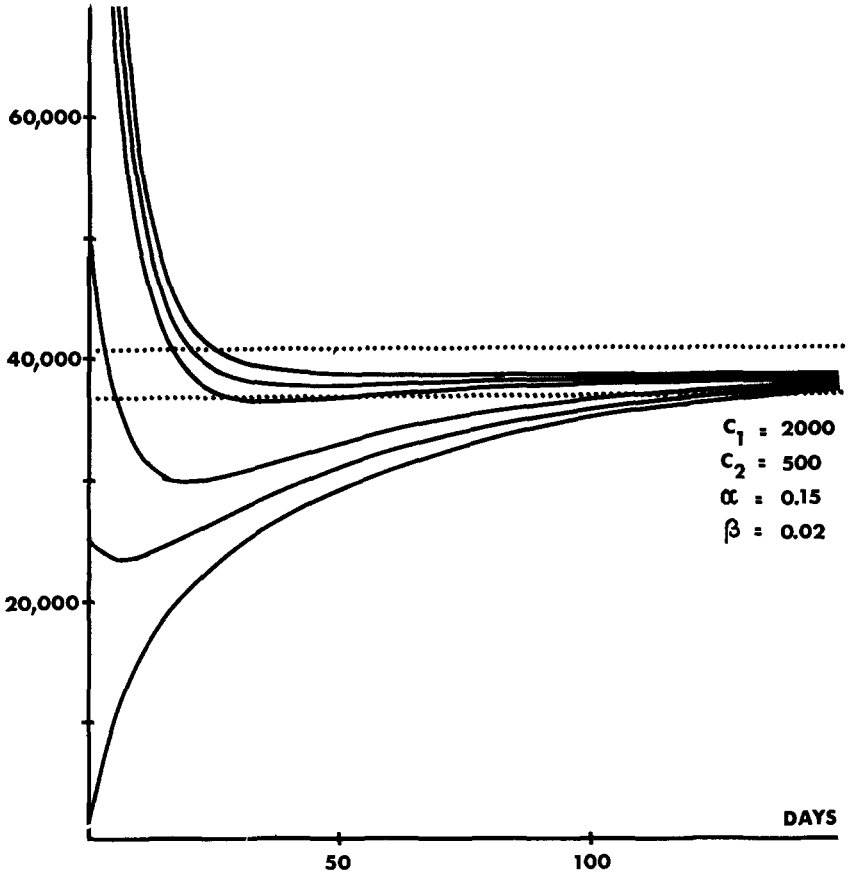

FIG. 1. Total label in compartment A, or plasma, after daily dosage of isotope combined with a single priming dose M times the size of the daily dose. Curves from top to bottom illustrate the time course of radioisotope concentrations for the following values of M: 50, 45, 40, 20, 10, 0. The numbers on the ordinate are arbitrary, but could represent radioactive units in compartment A, or per ml of plasma, or specific activities of plasma cholesterol. Dashed horizontal lines are at 105% (top) and 95% (bottom) of isotopic steady state.

the steady-state value, drops to 5% above steady state at 16.6 days, and continues to fall, reaching 5% below steady state at 28.3 days, and a minimum of 5.6% below steady state at 35 days; it does not rise above the 95% of steady-state value until 47 days. Thus at this priming dose, a plasma isotope concentration which stays within 6% of the steady state is reached at about 16 days, but takes three times as long to reach an isotope concentration which remains within 5% of that value. A slight increase in priming dose will give a curve that has a minimum value within 5% of the steady-state level. This happens at a priming dose of 41 times the daily dose. Here we find that 17 days after the beginning of the dosage schedule the plasma isotope concentration decreases to 105% of the steady-state value, then drops down to a minimum isotope concentration, equal to 95% of the steady-state value and from then on slowly rises towards the theoretical value for the steady state. At higher priming doses the time required to reach the steady state begins to increase again until at a priming dose of 100 times the daily dose, it takes 128 days to reduce the plasma isotope level to 105% of the equilibrium value. This is the same as the time required to reach the steady-state concentration when no priming dose is given. At even higher priming doses one does considerably poorer than is the case without a priming dose. Apparently, by the criteria set forth earlier, a priming dose of 41 times the daily dose is optimal.

From Table 1 it is also evident that the total dose of isotope administered to the patient can be reduced significantly by an optimal combination of priming and daily dose. At a priming dose 41 times the daily dose, the total isotope dosage used in the 17 days to reach the steady state is found to be 46% of the dose that would be required if no priming dose were used.

In the studies by Grundy and Ahrens (5) the daily dose of labeled cholesterol was administered by mouth, whereas the priming dose was given intravenously. If one wishes to apply the foregoing considerations for choosing an optimal priming dose one must take into account that while all of the priming dose is introduced into the rapidly exchanging pool (plasma, liver, etc.) only that por-

TABLE 1 ESTABLISHMENT OF THE ISOTOPIC STEADY STATE FOR DIFFERENT PRIMING DOSES

Priming Dose Factors (M)*	Minimum		Isotopic Steady State‡	
	days	Isotope Concentration	days	Total Isotope Dosage
0	—	—	128	100
10	7	61	116	98
20	20	77	102	95
40	35	94	45	66
41	*36*	*95*	*17*	*46*
45	42	98	21	52
50	—	—	26	59
100	—	—	128	179

* Priming dose expressed as multiples of daily dose. Italicized values are for the optimum priming dose.

† Isotope concentration in plasma expressed as a percentage of steady-state concentration.

‡ Isotopic steady state with 5% of the theoretical value. Total isotope dosage is expressed as a percentage of that for $M = 0$.

TABLE 2 OPTIMAL PRIMING DOSE FACTORS (M_{opt}) AND TIME OF EQUILIBRATION FOR DIFFERENT VALUES OF C_2 AND α

α \ C_2	100		500		1000		2000	
	M*	days†	M*	days†	M*	days†	M*	days†
0.30	40	13	45	10	45	8.5	46	6.4
0.15	27	19	41	17	43	14	44	10.6
0.075	15	0	32	18	37	17	40	11.8
0.030	32	0	35	0	37	0	40	0
0.30	48	19	49	16	49	14	49	12
0.15	44	33	48	29	48	26	48	22
0.075	30	46	43	47	45	42	47	36
0.030	34	0	37	0	39	3.6	42	45

* M is the optimal priming dose in multiples of the daily dose.

† Days required for plasma isotope concentration to reach within 5% (upper half) or 1% (bottom half) of steady-state concentrations. In all cases, $C_1 = 2000$ and $\beta = 0.02$. Note that in some instances steady-state concentrations may exist from the first day of the experiment.

tion of the daily dose which is absorbed enters this pool. In principle it is, therefore, preferable to administer the priming and the daily doses by the same route. The oral route has the advantage that, because of relatively slow cholesterol absorption from the gastrointestinal tract, the appearance of label in compartment A is more nearly continuous than would be the case for an intravenous dose. If for practical reasons one must administer the priming dose by vein and the constant dose by mouth, one should correct the optimal value for the priming dose, calculated theoretically, by a factor based on the efficiency of cholesterol absorption from the gastrointestinal tract.

One might compare the foregoing results with the situation obtained when only a single dose of label is employed. In that instance one waits until the log(radioactivity)–time relationship has become linear before one analyzes the curve into its component parts. For the case represented in Fig. 1 and Table 1, it would take about 35 days before the concentration of label in plasma would have fallen to within 5% of the straight line representing the final linear slope of the curve. In this situation, as well as in the situations discussed previously, one would, of course, have to proceed well beyond this minimum time interval in order to ascertain whether the straight line portion, or isotopic equilibrium as the case might be, had indeed been reached.

The foregoing considerations have been based on the assumption that the kinetics of cholesterol metabolism in man may be described adequately by a two-compartment model. Although the data of Goodman and Noble (1) provide evidence for this model, more recent studies (6) have shown that, when measurements are continued for approximately 1 yr after administration of a single dose of radioactive cholesterol, a third exponential shows up in many cases.

General Method for Determining Optimal Priming Doses

The example in Fig. 1 has employed values for C_1/C_2, α, and β which are reasonably representative for those found in the two-compartment model proposed for cholesterol metabolism by Goodman and Noble (1). Fig. 2 shows four sets of curves in which widely different values for these constants were selected. Table 2 lists the optimal priming dose and the time required to reach a plasma isotope concentration to within 5% or 1% of the theoretical steady-state values. It is apparent that the choice of an optimal priming dose (M_{opt}) depends upon the value of these constants.

It may be shown algebraically that the optimal value of M: (a) depends upon the ratio C_1/C_2 and not upon the numeric values of each of these parameters, (b) depends upon the numeric value of α and β, and (C) lies somewhere between $1/\alpha$ and $1/\beta$. Just where M lies between $1/\alpha$ and $1/\beta$ may be described by a weighting factor f:

$$M_{opt} = f\frac{1}{\beta} + (1 - f)\frac{1}{\alpha} \qquad \text{eq. (6)}$$

Like M_{opt} the factor f is dependent upon C_1/C_2 and, in addition, is uniquely determined by α/β. Values of f as related to C_1/C_2 and α/β have been computed for situations in which it is desired to reach the isotopic steady states ($\pm 5\%$ and $\pm 1\%$, respectively) at the earliest possible times. Figs. 3*A* and 3*B* allow the estimation of f, and therefore of the optimal priming dose M_{opt}, for any set of curves with C_1/C_2 between 0.5 and 200 and with α/β between 1 and 100.

Thus, to estimate the value of M_{opt} by this method, one needs approximate values for C_1/C_2 and of α and β. Using the appropriate graph, a point is located which has C_1/C_2 as its abscissa and α/β as its ordinate. From this point one reads the value of f from the plotted lines, by interpola-

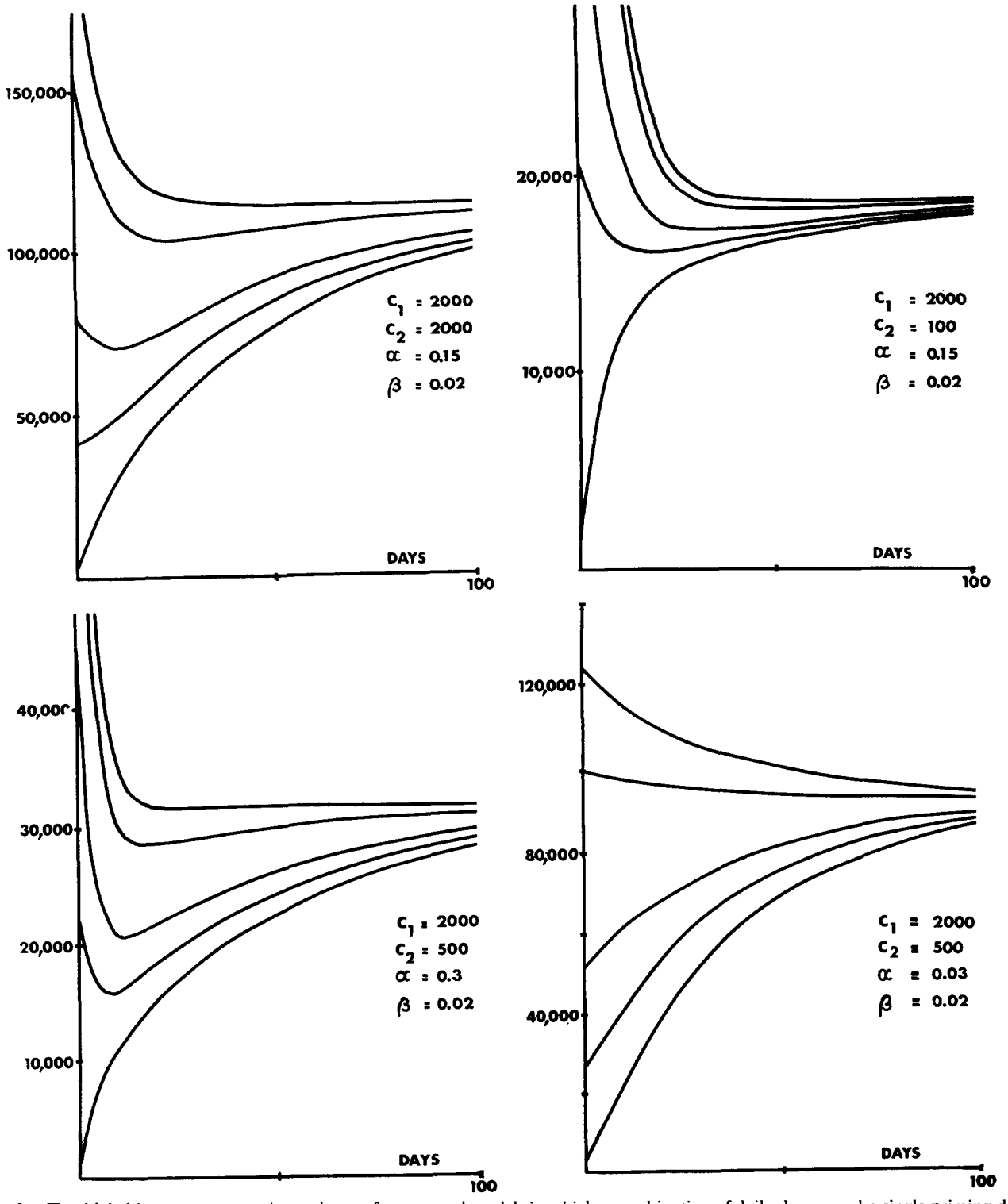

FIG. 2. Total label in compartment A, or plasma, for two-pool models in which a combination of daily dosage and a single priming dose are used. Values for M in each graph from top to bottom are: 50, 40, 20, 10, 0.

tion when necessary. Knowing f one calculates M_{opt} by means of eq. (6). The value of M_{opt}, multiplied by the daily dose, then gives the optimal priming dose.

In most of the two-compartment biological systems with which we are familiar, f would be closer to 1 than zero, that is, M_{opt} will not be greatly less than $1/\beta$. Thus, the importance of accuracy in estimates of values for system parameters in order to obtain experimentally usable values for M_{opt} diminishes in the following order: β, α, and, least critical, C_1/C_2.

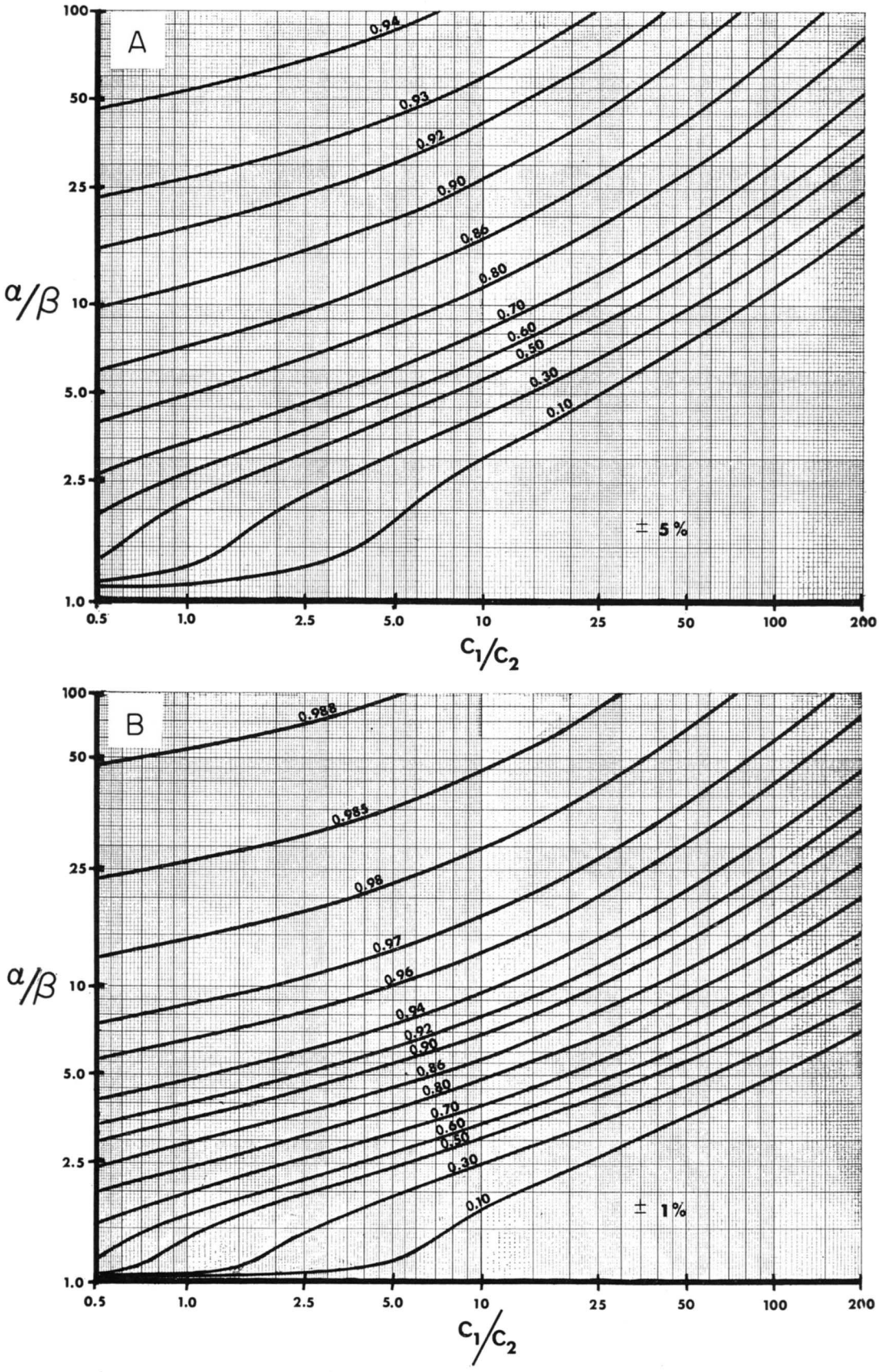

FIG. 3. Values of weighting factors f for the estimation of optimal priming doses (M_{opt}) by eq. (6). The value of f is read from the diagonal line nearest the point determined by the Cartesian coordinates of C_1/C_2 (abscissa) and α/β (ordinate). The top diagram (A) provides values of f to calculate the optimal priming dose, as a multiple of the daily dose, for achieving a plasma isotope concentration within 5% of the theoretical steady-state values. The lower diagram (B) gives values of f for the optimal priming dose which achieves plasma isotope concentrations within 1% of steady state in the shortest possible time.

The foregoing method allows the estimation of the optimal priming dose for a system in which the isotope concentration in plasma is described by a two-exponent equation. The method requires only approximate prior knowledge or estimates of the parameters of this equation. In many instances a sufficiently accurate optimal priming dose may be calculated based only on the estimated slope of the final semilogarithmic die-away curve.

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