# ERYTHROCYTIC PRODUCTION IN ANEMIAS. I. THE USE OF THE SURVIVAL CURVE FOR ESTIMATING PRODUCTION IN THE NONSTEADY STATE

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A red cell survival study is performed by periodic measurement of the concentration of labeled erythrocytes in the blood. The curve obtained containing the concentration of labeled cells per milliliter of red cells reflects the interplay of production and destruction as reflected by the loss of labeled cells and by the entry of newly produced unlabeled cells (*1*). The curve gives information about the rate of destruction only in the steady state (*2–4*); that is, when the total red cell volume remains constant because cell production and destruction are equal. It should be stressed that in this condition the curve also gives information on production rate which, together with a red cell volume, gives quantitative data on production in terms of volume of red cells produced per time unit (*5,6*).

In the nonsteady state the curve does not measure destruction nor production rate and should be considered simply as a label concentration curve. This curve, however, can give better insight into production and/or destruction in some situations in which a nonsteady state is present.

## THEORETICAL CONSIDERATIONS

The measurement of the destruction rate in the nonsteady state has been accomplished by serial red cell volume estimations (*3,7–9*). This procedure can give quantitative information on production and destruction as mean values for the period between each pair of red cell volume estimations. On the other hand, a label concentration curve obtained in the same period can give in some instances information on the behavior of the volume during this period and, consequently, on production and/or destruction at any given point of the label curve. This possibility can be seen in the fact that at any moment of a survival study, the total amount of label in the circulation can be obtained by two procedures:

1. Multiplying the label concentration C at time t by the red cell volume V present at that time.
2. Multiplying the initial label concentration $C_0$ by the remainder of the initial volume $V_r$ present at time t. That is,

$$CV = C_0V_r \qquad (1)$$

and consequently

$$V = \frac{C_0V_r}{C} = \frac{V_r}{C}, \text{ as } C_0 = 1. \qquad (2)$$

Equation 2 shows the way in which the values C of a label concentration curve together with $V_r$ can give data on red cell volume behavior.

In the performance of serial estimations of red cell volume, every two red cell volumes will give, among other data, an experimental $V_r$ for the period covered by the two estimations:

$$V_r = V_0\frac{C_fV_f}{C_0V_0} = \frac{C_fV_f}{C_0} = C_fV_f, \text{ as } C_0 = 1 \qquad (3)$$

in which $V_0$ and $V_f$ are the initial and final volume of the observation period, respectively, and $C_0$ and $C_f$ are the label concentration present at those specific times.

If, in addition, the type of destruction, random or senescent, can be established or surmised, a series of V values for that period can be calculated with Eq. 2 from the experimental $V_r$ and from the label concentration curve to obtain volume behavior and ultimately production behavior.

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## PROCEDURES

The most simple situation occurs when the destruction rate is constant so that the procedure requires only two red cell volumes. Section I deals with this situation.

**Section I:**

**Cell loss due to senescence.** The new cells produced from the start ($t_0$) to the end ($t_f$) of the observation period will not be destroyed if $t_f$ is smaller than the mean life span of the new RBC. By definition, the volume of red cells destroyed per time unit (D) will not vary and its value is given by

$$D = \frac{V_0}{t_f}\left(1 - \frac{V_r}{V_0}\right). \quad (4)$$

This equation is derived from that used in the steady state:

$$D = P = \frac{V_0}{t}\left(1 - \frac{C}{C_0}\right). \quad (4a)$$

Taking into account the cell volume changes, $C/C_0$ is substituted by $C_fV_f/C_0V_0$, that is, $V_r/V_0$ (see Eq. 3).

After D, a series of cell volume values (V) may be obtained,

$$V = \frac{C_0\,(V_0 - Dt)}{C}, \quad (5)$$

in which t are the time units which have elapsed since the start of the study. This equation is basically Eq. 2 since in this situation $V_0 - Dt = V_r$.

With the values of V determined, the corresponding values of production (P) can be calculated.

$$P = V - V' + D, \quad (6)$$

in which V′ is the value of V at the time unit preceding that for which P is determined.

**Cell loss due to rapid* random destruction.** In this situation the newly formed cells are destroyed at the rate in which the initial cell volume is being destroyed so that the volume of cells destroyed per time unit D will vary according to the cell volume. The fraction k of the initial volume destroyed per time unit is calculated from $V_r$ by the equation

$$k = \frac{2.303}{t_f}\log_{10}\frac{V_0}{V_r}. \quad (7)$$

Equation 7 is basically the accepted one for the steady state,

$$k = \frac{2.303}{t}\log_{10}\frac{C_0}{C}, \quad (7a)$$

which becomes Eq. 7 by substituting $C_0/C$ by $C_0V_0/C_fF_f$, that is $V_0/V_r$ (see Eq. 3). Equation 7 can be used with the raw data of autologous labeled studies; in isologous studies $V_r$ and the label concentration curve are corrected for senescent loss unless cohort labeling is used.

The series of cell volumes are computed with Eq. 2, and the values $V_r$ used in it are obtained from

$$V_r = V_0e^{-kt} \quad (8)$$

which is derived as follows: the equation describing the survival curve in the steady state is

$$C = C_0e^{-kt}, \quad (8a)$$

and more generally in the nonsteady state it can be expressed as

$$CV = C_0V_0e^{-kt}. \quad (8b)$$

In the steady state, $V = V_0$ and Eq. 8a is obtained. In the nonsteady state, Eq. 8b should be applied, but as stated in Eq. 1, $CV = C_0V_r$. By substituting Eq. 1 in Eq. 8b we have $C_0V_r = C_0V_0e^{-kt}$ which becomes Eq. 8 by dropping $C_0$.

With the value of V at any moment of the study, the corresponding D and P may be calculated from

$$D = kv \quad (9)$$

$$P = -\frac{V\,dC}{C\,dt} \quad (10)$$

in which dC/dt is the derivative of the label concentration curve with respect to time at any time t.

Equation 10 is derived from equations that can be generalized to any situation in which a volume $C_0V_0$ is labeled at time $t_0$ and the label concentration curve is known. If P and D are production and destruction of cells per unit time, respectively, the following equations can be written

$$V = V_0 + \int_0^t (P - D)\,dt \quad (10a)$$

$$CV = C_0V_0 - \int_0^t D^*\,dt \quad (10b)$$

in which D* is the volume of labeled cells destroyed per time unit. By differentiating Eqs. 10a and 10b we have

$$dV = (P - D)\,dt \quad (10c)$$

$$CdV + VdC = -D^*\,dt. \quad (10d)$$

Multiplying Eq. 10c by the label concentration C and subtracting it from Eq. 10d we have:

$$VdC = -[C(P - D) + D^*]\,dt,$$

that is

* A rate of 0.035%/day would kill practically all cells before reaching senescence; half of the population would die within 20 days of entering the circulation and only around 10% would reach 50 days of age.

$$\frac{dC}{dt} = -\frac{C(P - D) + D^*}{V}. \qquad (10e)$$

In the treated situation, the destruction of labeled cells is given by

$$D^* = CD = C\,kV \qquad (10f)$$

and that of all cells, labeled and unlabeled, by Eq. 9. Substituting Eqs. 9 and 10f in Eq. 10e, we have

$$dC/dt = -CP/V \qquad (10g)$$

which is Eq. 10 when solved for P.

**Cases of constant production.** There are situations in which the nonsteady state is a consequence of variations in rapid random destruction rather than in production, i.e., in the initial response of an autoimmune hemolytic anemia to steroid therapy. In these cases the quantitation of destruction can be performed with two cell volumes estimated at short intervals (hours or a couple of days), that is, before changes in red cell volume due to the decrease in destruction induce changes in production.

The constant P is computed from Eq. 10, from the label curve at the point in which V was actually measured, i.e., at time $t_0$ (with $V_0$) or $t_f$ (with $V_f$) using the right side derivative for $V_0$ or the left side derivative for $V_f$.

After P, the red cell volume at any point of the label concentration curve can be obtained by:

$$V = -PC\frac{1}{dC/dt}. \qquad (11)$$

Equation 11 is Eq. 10g when solved for V. It can be used with the raw data of autologous studies; with isologous cells (noncohort labeling) the label concentration curve must be corrected for senescent loss.

A curve of the variable k can be plotted with the following equation

$$k = \frac{1}{V}\left(P - \frac{dV}{dt}\right) \qquad (12)$$

in which dV/dt is the derivative of the V curve with respect to time at any time t. Equation 12 is obtained by substituting Eq. 9 in Eq. 10c,

$$dV = (P - kV)dt, \qquad (12a)$$

which is Eq. 12 when solved for k.

Finally, the volume of erythrocytes destroyed at different times can be obtained with Eq. 9 using the values V and k obtained from Eqs. 11 and 12.

The situations in which production is the constant and senescent destruction is the variable should be treated in the manner described in Section II.

**Section II**

**Varying rates of production and destruction.** In the cases where both rates, production and destruction, are changing, the study requires serial determinations of red cell volume. With these cell volumes a volume curve is plotted as a function of time. This curve associated with the label concentration curve will give production and destruction by the following calculations:

**for senescent destruction:**

$$D = -\frac{1}{C_0}\left[V\frac{dC}{dt} + C\frac{dV}{dt}\right] \qquad (13)$$

$$P = \left(1 - \frac{C}{C_0}\right)\frac{dV}{dt} - \frac{V}{C_0}\frac{dC}{dt}. \qquad (14)$$

The derivation of Eqs. 13 and 14 is based on Eqs. 10c and 10d.

Solving Eq. 10c for P:

$$P = \frac{dV}{dt} + D. \qquad (13a)$$

Solving Eq. 10d for D*:

$$-D^* = V\frac{dC}{dt} + C\frac{dV}{dt}. \qquad (13b)$$

Also:

$$D = \frac{D^*}{C_0}, \qquad (13c)$$

because the number of cells labeled and unlabeled that will die at a certain time is proportional to the labeled fraction attaining its life span. Substituting Eq. 13b in Eq. 13c we have Eq. 13.

Introducing Eq. 13 in Eq. 13a we have

$$P = \frac{dV}{dt} - \frac{1}{C_0}\left[V\frac{dC}{dt} + C\frac{dV}{dt}\right] \qquad (14a)$$

which gives Eq. 14 by simplification.

**for destruction due to rapid random loss**

$$k = -\left[\frac{dC}{Cdt} + \frac{dV}{Vdt}\right]. \qquad (15)$$

$$P = -\frac{V}{C}\frac{dC}{dt}. \qquad (10)$$

Equation 15 is obtained by substituting Eq. 10f in Eq. 10d; this gives the expression $C\,dV + V\,dC = -C\,kV\,dt$, which divided by CV renders

$$\frac{dV}{V} + \frac{dC}{C} = -k\,dt \qquad (15a)$$

which is Eq. 15 when solved for k. When donor cells (noncohort labeling) are used, the label concentra-

tion curve is corrected for senescent loss before using Eqs. 10 and 15.

## COMMENTS

It should be noted that the sets of equations given in Section I are appropriate for populations in which all cells have the same potential life span, i.e., a homogenous population. In dealing with populations having different life spans, two situations may arise: (A) all life spans are longer than the period of observation; if so, the data can be handled as that of a homogenous population; (B) the life span of at least one of the populations is shorter than the period of observation; in this case it should be handled as described in Section II.

No procedure has been given in this paper for the situation in which random destruction is slow so that senescence is operating on a significant proportion of the cell population. In this case, two requirements, difficult to establish, would have to be met: (A) the random destruction must have been present before the start of the observation period for a lapse at least equal to the potential life span of the cell population; and (B) the production rate must have remained constant before the start of the observation period.

Ideally, all cases should be studied as described in Section II so that no assumptions on the constancy of destruction or production rate have to be made. On the other hand, there appear to be in the literature a sizable number of cases in a nonsteady state in which only two cell volume estimations in addition to a label concentration curve are available, and the equations given in Section I may permit their analysis.

The use of serial estimations of cell volume in Section II could be improved by monitoring the cell volume although as yet there is no available method. Nevertheless, the serial estimations, if spaced close enough, give good approximations, and the procedures presented in this paper may render valuable information on erythrocytic kinetics in spite of this shortcoming.

## SUMMARY

The paper gives the basis and the mathematical formulation needed to measure erythrocytic production and destruction in some situations in which a nonsteady state of erythropoiesis is present. To obtain quantitative data on the kinetics of erythropoiesis during the lapse of study, the equations demand basically two or more red cell volume estimations associated with the changes in the concentration of labeled cells per milliliter of cells occurring during the time that elapses between each pair of red cell volume estimations. The approach is applicable to cases in which the cell loss is due mainly to a single mechanism of destruction, either senescence or random.

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