

# Estimation of the Life Span of Red Blood Cells

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**ABSTRACT** A modified method for fitting the model of Shemin and Rittenberg to study the life span of red cells is described. In this modification the assumption that incorporation of tag is complete before any cells die is relaxed. A recursion formula useful for fitting the model on the digital computer is given. Good results were obtained with data from the sheep, rat, cat, rabbit, and the dog, whereas in several of these cases the method of Shemin and Rittenberg was not satisfactory. By varying critical parameters of the model a continuum of curves is possible. Curves which formerly were explained on the basis of random death can be obtained without this assumption. Limitations and implications of the method are discussed.

## INTRODUCTION

Several different methods have been used to estimate the average life span of red blood cells. Most of these methods can be put into two general classes: those which "tag" the cells as they are being formed, and those which attempt to tag proportionately the cells of all ages found in the vascular system.

In both classes of methods, the concentration of "tagged" red cells in the blood is followed for a sufficiently long period of time. Parameters of a mathematical model supposed to express the concentration of tagged cells as a function of time are then estimated from the data. Ordinarily one of these parameters represents the average life span.

The present study deals with the use of the method of tagging the newly forming cells. In particular, attention is directed to the assumption made in the fitting of the model of Shemin and Rittenberg (1).

Difficulties were encountered when using the method of Shemin and Rittenberg in a series of experiments being conducted to determine the "physiological requirement" of iron (2-4), the effect of copper deficiency

(5), and the effect of zinc-induced copper deficiency (6) in erythrocyte formation for several species of animals. The present paper reports how these difficulties were overcome and certain associated implications.

#### MATERIALS AND METHODS

To demonstrate the difficulties and how they were overcome data from three normal wethers approximately 2 years of age, ranging from 155 to 172 pounds in weight, were used. Each sheep was injected intravenously with a single dose of 100  $\mu$ c of glycine labeled with  $C^{14}$  in the methylene carbon. Twenty ml samples of blood were collected from the sheep at various intervals following injection, and hemin was isolated using the method described by Shemin and Rittenberg (7). Details of the biological phases have been published elsewhere (8). The specific activities observed in the hemin of the three sheep are indicated in the first three curves of Fig. 2 under Results.

The successfulness of the modified method of fitting is illustrated with published data on four other species of animals.

#### THE MODEL AND METHOD OF FITTING

Shemin and Rittenberg found that glycine was utilized for hemoglobin formation and remained as an integral part of the hemoglobin with no measurable metabolic exchange as long as a red cell was intact. Isotopic glycine could thus be used as a tag to study life span, and for this purpose they proposed a model which has been used widely.

The Shemin and Rittenberg model is

$$C(t) = \rho \int_{\theta=0}^t f(\theta)\phi(t-\theta) d\theta \quad (1)$$

where

$C(t)$  = concentration of tag in hemin at time,  $t \geq 0$ ;  $t = 0$  at the time of administering the tagged precursor.

$\rho$  = a proportionality constant;  $\rho$  is partially associated with the rate of formation of red blood cells.

$f(\theta)$  = concentration of tag in the hemin synthesized at time,  $\theta$ ;  $0 \leq \theta \leq t$ ;  
 $f(\theta)$  is proportional to the concentration of the tagged precursor at time  $\theta$ .

$\phi(t) = 1 - F(t)$  = probability that a cell formed at time,  $t = 0$ , will survive until time,  $t$ ;  $\phi(t)$  is the cumulative survival distribution of the cells; and  $F(t)$  is the cumulative mortality distribution.

Thus,  $\phi(t - \theta)$  = the probability that a cell formed at time,  $\theta$ , survives until time,  $t$ .

Knowledge of  $\phi(t)$  or  $F(t)$  supplies complete information on the distribution of length of life of red blood cells, including average length of life, variance of length of life, etc. Since neither  $\phi(t)$  nor  $f(\theta)$  is known, certain simplifying assumptions must be utilized in order to express the right-hand side of (1) in a form which permits fitting to experimental data on  $C(t)$ . The validity of the analysis is subject to these assumptions.

Three assumptions of import were made by Shemin and Rittenberg to obtain a solution for their data, which were obtained on human subjects. The first assumption was that

$$f(\theta) = \alpha e^{-\lambda\theta} \quad (2)$$

where  $\alpha$  is a constant determined primarily by the dilution of the tagged precursor in the precursor pool at  $t = 0$  and  $\lambda$  is determined almost entirely by metabolism of the precursor for processes other than hemoglobin formation. The second assumption was that the mortality distribution was a normal one; this can be stated as follows:—

$$F(t) = \frac{1}{\sqrt{2\pi}\sigma^2} \int_{-\infty}^t e^{-\frac{(\tau - \mu)^2}{2\sigma^2}} d\tau, \quad (3)$$

where

$\mu$  = mean time to death.

$\sigma^2$  = variance of time to death.

The third assumption was that  $\mu$  is great enough and  $\sigma^2$  is small enough so that deaths during the incorporation phase can be disregarded. That is,  $f(\theta)$  becomes negligibly small before  $F(t - \theta)$  becomes large enough to be important.

The third assumption was made for convenience in fitting the data, because an explicit solution (1) does not exist when (2) and (3) are used. The assumption states essentially that

$$-\frac{d\phi}{dt} = \frac{dF}{dt} \propto -\left\{ \frac{1}{\lambda} \frac{d^2C}{dt^2} + \frac{dC}{dt} \right\} \quad (4)$$

## RESULTS USING THE METHOD OF SHEMIN AND RITTENBERG

### *Sheep Data*

The three assumptions worked well for the data of Shemin and Rittenberg. In the present study they were satisfactory for two of the sheep, B and C, but not for the third, sheep A (for data from the three sheep see Fig. 2 below). The unsatisfactory fit for sheep A was found to be attributable to a failure of the third assumption to hold. That is,  $f(\theta)$  remained substantial until after  $F(t - \theta)$  became substantial. This is explainable in terms of (4), as follows: If  $\lambda$  is small enough, the term  $(1/\lambda) \frac{d^2C}{dt^2}$  may be large enough over some range of  $t$  to render the mortality density function,  $dF/dt$ , negative.

Negative  $dF/dt$  values were in fact obtained (Fig. 1) by us in attempting to use Shemin and Rittenberg's method on the data from sheep A. This curve was obtained using  $\lambda = 0.062$  from a fit of the initial part of the data.

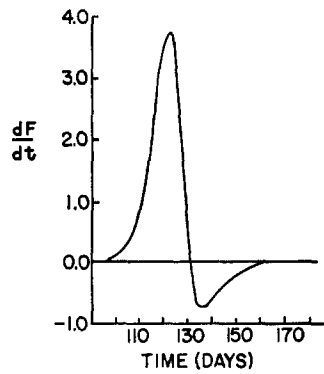


FIGURE 1. Density distribution  $dF/dt$  plotted against time. Obtained from data of sheep A.

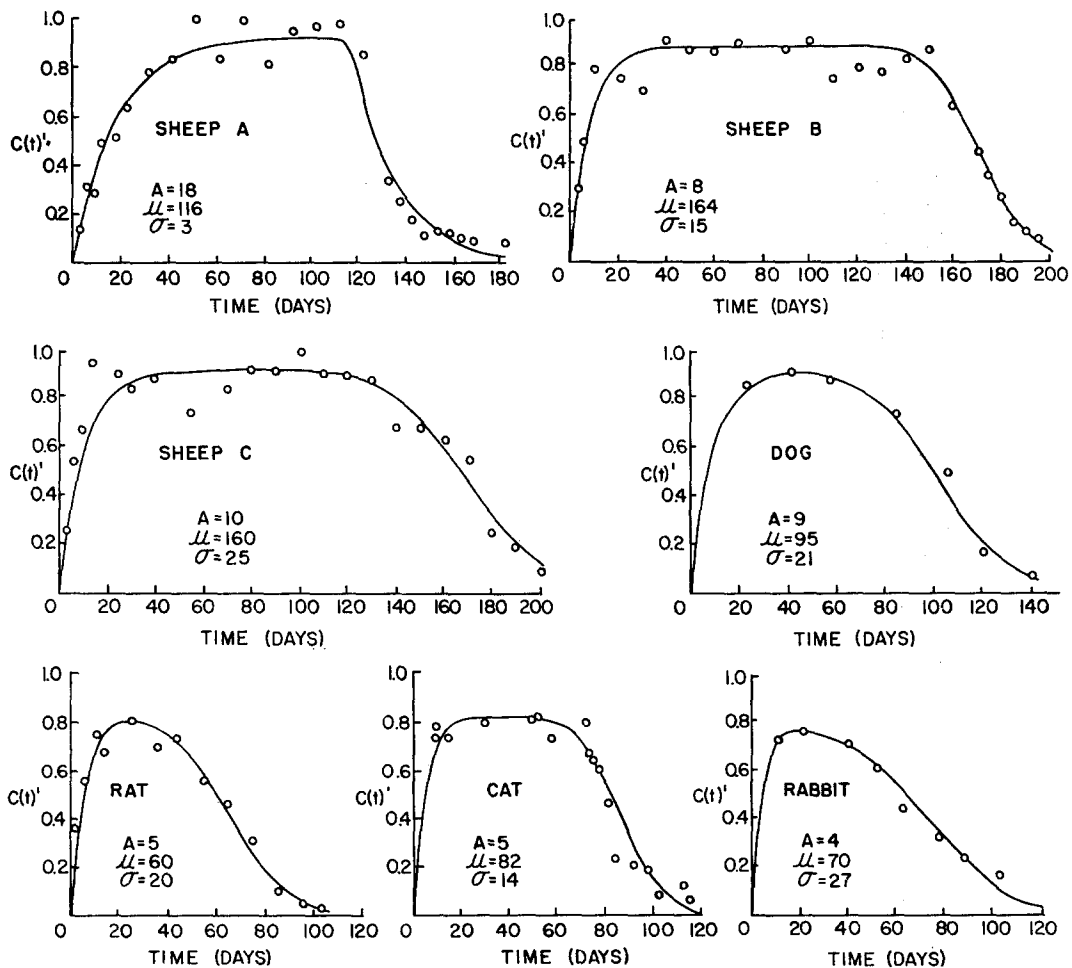


FIGURE 2.  $C(t)'$ , which equals the observed specific activity times the proportionality constant  $1/\gamma$ , plotted against time for the sheep, rat, cat, rabbit, and dog. The proportionality factors for sheep A, B, and C are 0.020, 0.023, and 0.022, respectively.  $A = 1/\lambda$ .

An arbitrary value of  $\lambda = 0.123$  was sufficiently large to result in non-negative  $dF/dt$  over the whole range of  $t$ ; however, this latter value of  $\lambda$  was not consistent with the data obtained during the incorporation phase.

In order to take care of this problem, the assumption that incorporation and mortality did not overlap significantly was relaxed. That is, it was decided to fit (1) directly, after substitution of (2) and (3), by numerical means, subject only to the restriction that  $\mu/\sigma > 2.5$ . This restriction was made so that  $F(0)$  would be negligible; actually  $F(0) \simeq 0.006$ .

A program to generate  $C(t)$  for various choices of the parameters,  $\gamma$  ( $\gamma = \rho\alpha$ ),  $\lambda$ ,  $\mu$ , and  $\sigma$ , was written for a digital computer. The numerical integration was accomplished using the following recursive relationship:

$$C(t) = \gamma \sum_{\tau=0}^t Q_{\tau} P\left(Z > \frac{t - \tau + \frac{1}{2} - \mu}{\sigma}\right)$$

where

$\gamma$  = proportionality constant.

$\tau = 0, 1, 2, \dots, t(\text{days})$

$$Q = k \int_{\tau}^{\tau+1} e^{-\lambda\theta} d^{\theta}$$

$$P(Z > Z_i) = 1 - F(t)$$

The observed data and the theoretical curves which were obtained by iteration on  $\gamma$ ,  $\lambda$ ,  $\mu$ , and  $\sigma$  are shown in Fig. 2 in which  $C(t)' = C(t)/\gamma$  is plotted against  $t$  and  $A = 1/\lambda$ . Except for short term aberrations the fits are good. Note that  $A(1/\lambda)$ ,  $\mu$ , and  $\sigma$  all differ to some extent among the three sheep. The most marked feature, however, is that  $A$  is larger and the other two parameters are smaller for sheep A than for sheep B and C. In biological terms it may be stated that the labeled precursor pool persisted for a longer period of time, the average life span was shorter, and the variation in life span was less for sheep A than for the other sheep. Although the metabolic events causing these differences cannot be ascertained from this kind of experiment, the significant point is that the model contains three potentially meaningful parameters.

#### *Data on Other Species*

The modified method of fitting was used on data from the literature for the rat (9), the cat (10), the rabbit (11), and the dog (12). In each of these studies tagged glycine was used. The results are shown in Fig. 2. The fits are good.

## DISCUSSION

It is apparent from the different type curves obtained in the sheep, rat, cat, rabbit, and dog that a variety of forms may be realizable by the model. By varying critical parameters of the model other forms were obtained. These may be seen in Fig. 3. From this figure it may be seen that the lack of a plateau is completely consistent with the model. In addition, where the

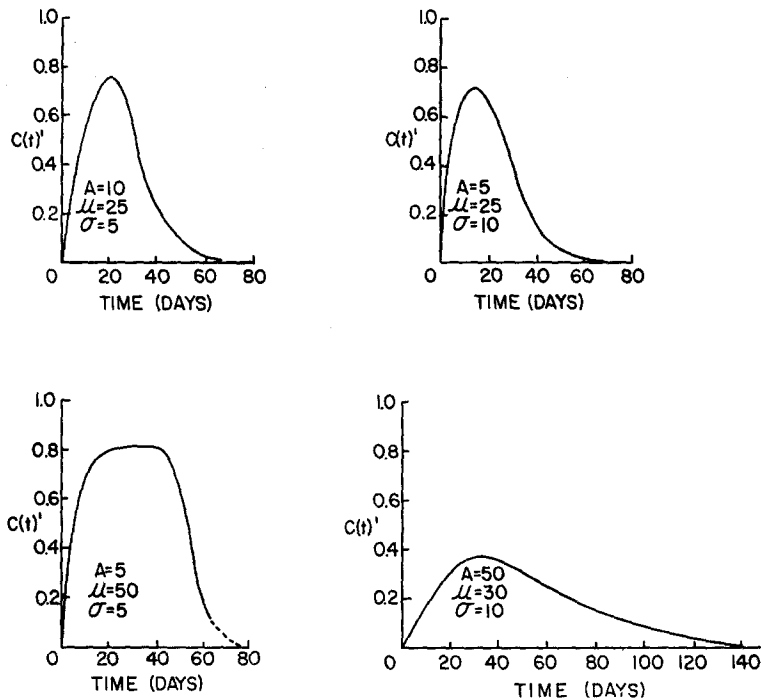


FIGURE 3. Theoretical curves obtained from model illustrating the effect of changes in the parameters  $A$  ( $1/\lambda$ ),  $\mu$ ,  $\sigma$  on concentration of tag as related to time.

plateau is absent rounded peaks of varying sharpness occur. These peaks can skew either right or left. In addition to this plateaus of various lengths are possible. However, it is not possible to describe "plateaus" which are sloping with this model unless a graph is obtained by plotting  $\log C(t)$  vs.  $t$ .

Generally the absence of the plateau has been interpreted as meaning that there is random death of the red blood cell as well as an age-dependent process. Since, as indicated previously, the absence of a plateau is consistent with the model which does not include random death it is apparent that although random death would result in the absence of a plateau, the converse is not necessarily true; that is, that the absence of a plateau implies random death. The model proposed by Brown and Eadie (13) was also considered.

However, the assumption is made in obtaining the model that all the tagged cells are formed instantaneously or in a very short interval of time. It should be realized in this case that it is possible to obtain a coefficient associated with random death in the model purely as a result of the lack of instantaneous formation when no random death is present. In addition to this where the plateau is present, and hence negligible random death, the incorporation of tag over a long interval of time could appreciably bias the estimates when the method of Brown and Eadie is used. That incorporation may occur over prolonged intervals of time is apparent from the data of sheep A (Fig. 2).

The method described herein is limited to situations in which there is little or no reincorporation of the tag (1). Although this is true for the case reported herein in which tagged glycine was used, this may not be true in general. Thus if radioactive Fe was used the assumption of negligible reincorporation would not be met. The modification of the model to provide for reincorporation of tag will lead to a much more complex model.

A comparison of the values of the parameters of the model among the species considered herein indicates that the primary differences occur in the values of  $1/\lambda$  or  $A$  and  $\mu$ . The value of  $\lambda(1/A)$  was higher in the smaller animal than in the larger animal, indicating that the rate of disappearance of the tag available for incorporation was faster in the smaller animal. This is an indication that  $\lambda$  may be associated with the general metabolic rate of the animal. The reason for the shorter life span is somewhat more obscure. In the cases reported here it is probably related to genetic differences; however, it is not clear just what physiological process the genetic differences are affecting. Other factors which may affect the value of the parameters are nutrition, stress conditions, and disease.

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