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STOCHASTIC APPROACH
TO THE PROCESS OF RED CELL DESTRUCTION

1. Introduction. The examination of the rate of erythrocyte destruction is an important element in the diagnosis of haemolytic anaemias. Red blood cells labelled by some radioisotopes are used in this procedure. The methods which are used fairly widely in clinical practice are those of "random labelling" in which a sample of the whole population is labelled [4]. The simultaneous control of the circulating red cell volume enables to perform the determination of erythrocyte survival also in so-called non-steady states, i.e. in the cases in which the lack of equilibrium between erythrocyte production and destruction is observed ([6], [8], [10], [11]).

In order to interpret the red cell survival data several mathematical models have been proposed ([3], [8], [9], [12], [13]). In describing results observed, most researchers have until now used a single index: the time taken for half of the labelled erythrocytes to leave the circulation (i.e. to die) or the mean red-cell life span. Nevertheless, erythrocytes are destroyed simultaneously by senescence and random destruction, so it is recommended to determine separately the indices of both these processes [4], i.e. the erythrocyte potential life span T and the coefficient of their random destruction K. The analysis of a broad spectrum of erythrocyte survival data obtained in patients with haemolytic anaemias, observed both in steady and non-steady states [7], allowed us to distinguish several variants of erythrocyte survival patterns ([5], [11]). Erythrocyte kinetic characteristics presented in papers [7], [9] and [11] are sufficient to interpret all of them. Nevertheless, the trials of approximation of experimental data by theoretical survival curves revealed excellent accordance in some cases and marked discrepancies in others (all data at our disposal were obtained with the aid of the same instruments and methods). The problem arises whether only measurement errors are the reason of these discrepancies or perhaps also a marked statistical dispersion of the process of erythrocyte destruction. The aim of this work was to analyze the second possibility. It is obvious that the ideas used here may be applied to describe more general variants of cell behaviour [2].

2. The model. The mathematical models of erythrocyte destruction, known in the literature (see [8] and [12]), consist of equations describing the mean value of cell count. Such an approach supplies no information on the dispersion of the process considered. In this section we give a probabilistic description of our model in terms of a simple Markov process with continuous time (see Fig. 1). The formulae obtained for variances are used in the discussion of a fitting procedure described in the next
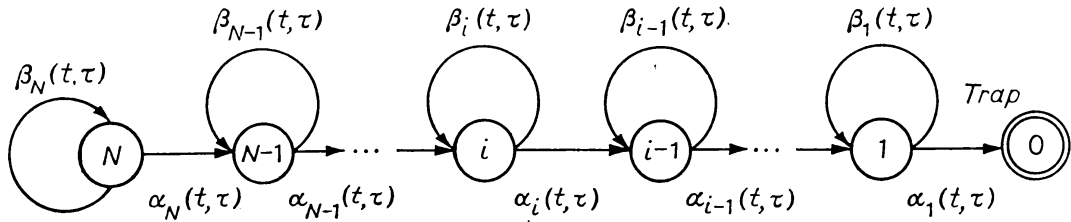

Fig. 1. Destruction of erythrocytes as a Markov process. The trapping state 0 is achieved at finite time less than or equal to T

$$a_i(t, \tau) = i\left[K + \frac{f(t)}{1 - F(t)}\right]\tau, \qquad \beta_i(t, \tau) = [1 - iK\tau]\left[1 - i\frac{f(t)}{1 - F(t)}\,\tau\right]$$

section (see also [5]). In what follows it is assumed that we have at our disposal a sample of identical red cells being independent one of another. The cells are labelled at time $t = 0$ and may die by two reasons:

(a) the age a of the cell is equal to T,

(b) the cell is randomly "shot" (in the time interval $[t, t+\tau]$ with probability $K\tau + o(\tau)$, where K is the coefficient of random destruction and $o(\tau)/\tau \to 0$ as $\tau \to 0$).

The age structure of the red cell population in blood is assumed to be described by the age distribution density $g(a)$, $a \in [0, T]$, such that

$$\int_0^T g(a)\,da = 1$$

and the fraction of cells in age from the interval $[a, a + \Delta a]$ is equal to $g(a)\Delta a + o(\Delta a)$. Therefore, for a cell in the randomly labelled sample, the probability of death by reason (a) before time t ($t \in [0, T]$) is equal to

(1) $$F(t) = \int_0^t g(T - a)\,da.$$

Define additionally $f(t) = dF/dt = g(T - t)$. Then the probability of death by reason (a) in $[t, t+\tau]$ under the condition that it did not occur up to the moment t is equal to

$$\frac{f(t)}{1 - F(t)}\,\tau + o(\tau).$$

It is assumed that the probability of simultaneous random and "natural" deaths, both of them being independent, is equal to $o(\tau)$ and that the count of cells labelled at $t = 0$ is given and equal to $n(0) = N$. The process of labelled red cells destruction is then the pure death process [2] with time-dependent death rate, described by the system of Kolmogorov differential equations

$$\dot{P}_n(t) = [-nP_n(t) + (n+1)P_{n+1}(t)]\left[K + \frac{f(t)}{1 - F(t)}\right], \qquad n = 0, 1, \ldots, N-1,$$

(2)

$$\dot{P}_N(t) = -NP_N(t)\left[K + \frac{f(t)}{1 - F(t)}\right],$$

where $P_n(t)$ denotes the probability that n cells are still alive at time t. Solutions of system (2) with usual initial conditions $P_N(0) = 1, P_n(0) = 0$, $n = 0, 1, \ldots, N-1$, may be obtained, e.g., from Theorem 2.3 (C) in [2], where a more general case is considered. In our notation

$$P_n(t) = \begin{cases} \binom{N}{n} e^{-nKt}[1 - F(t)]^n \{1 - e^{-Kt}[1 - F(t)]\}^{N-n}, & t \in [0, T], \\ P_n(T), & t > T. \end{cases}$$

For practical reasons it is enough to use the first two moments of $P_n(t)$ which are obtained from known relations for the binomial distribution, i.e.

(3) $\qquad \mathbf{E}[n(t)] = NA(t), \qquad \mathbf{D}^2[n(t)] = NA(t)[1 - A(t)],$

where

(4) $\qquad A(t) = [1 - F(t)]e^{-Kt}\mathbf{1}(T - t), \qquad t \geqslant 0,$

and $\mathbf{1}(\cdot)$ — the Heaviside step function — equals 0 for negative and 1 for non-negative arguments, $F(t) = 1$ for $t \geqslant T$. Note that the dispersion ϱ of the process,

(5) $\qquad \varrho(t) = \dfrac{\sqrt{\mathbf{D}^2(n)}}{\mathbf{E}(n)} = \sqrt{\dfrac{1}{N}\left(\dfrac{1}{A} - 1\right)}, \qquad t \in [0, T),$

s an increasing function of t. Table 1 contains values of ϱ for several combinations of values of A and N.

Assuming the simplest case of the uniform age distribution $g(a)$ of the red cell population in blood (i.e. $F(t) = t/T, t \in [0, T]$) we obtain

(6) $\qquad \mathbf{E}[n(t)] = N\dfrac{T-t}{T} e^{-Kt}\mathbf{1}(T - t), \qquad t \geqslant 0,$

as a survival pattern. This formula is mentioned in the literature (see [8]) but actually considered to be not satisfactory. We show that, in fact, it is based on the false assumption of uniformity of the age distribution.

TABLE 1. Values of dispersion ϱ for several combinations of A and N (see (5))

N	$A(t)$			
	0.5	0.1	0.01	0.001
10^2	0.1	0.3	0.99	3.20
10^5	0.032	0.055	0.31	1.0
10^8	0.0001	0.0003	0.00099	0.0032

To obtain the proper age distribution write the well-known ([1] and [12]) von Förster equation for the age distribution of the whole red cell population:

$$(7) \qquad \frac{\partial \mu}{\partial a} + \frac{\partial \mu}{\partial t} = -K\mu(a, t), \quad t \in [0, \infty), \ a \in [0, T],$$

where $\mu(a, t)\,da$ denotes the mean count of cells in age from the interval $[a, a+da]$ at the moment t. The production of cells is described by the boundary condition

$$(8) \qquad \qquad \mu(0, t) = S_0$$

and assumed to be constant and in equilibrium with the loss of cells $K\mu(a, t)$, where K is defined at the beginning of this section. The solution of (7), (8) is

$$\mu(a, t) = S_0 e^{-Ka}[1 - \mathbf{1}(a-t)] + h(a-t) e^{-Kt}\mathbf{1}(a-t),$$

where $h(a) = \mu(a, 0)$. The steady state $(t \to \infty)$ solution is of the form

$$\tilde{\mu}(a) = \mu(a, \infty) = S_0 e^{-Ka}.$$

We can normalize $\tilde{\mu}$ dividing it by V_0, the total count of cells in the population, obtaining the age distribution density

$$g(a) = \frac{\tilde{\mu}(a)}{V_0} = \frac{\tilde{\mu}(a)}{\int\limits_0^T \tilde{\mu}(a)\,da} = \frac{Ke^{-Ka}}{1 - e^{-KT}}, \quad a \in [0, T].$$

By substitution of $g(a)$ into (1) we get

$$F(t) = \int\limits_0^t g(T-a)\,da = \frac{e^{K(t-T)} - e^{-KT}}{1 - e^{-KT}}, \quad t \in [0, T],$$

whence

$$\text{(9)} \qquad \text{E}\,[n(t)] \; = \; N\,\frac{e^{K(T-t)}-1}{e^{-KT}-1}\,1(T-t), \qquad t \geqslant 0.$$

This last formula is the actually accepted survival pattern [7]. Quite analogous evaluations, but only for the mean values, are contained in [8]. Our evaluation could be also deduced from [12] but it is not stated there in the explicit form.

Formula (4) can be easily generalized onto the case where the coefficient of random destruction is a function of time (i.e. $K = K(t)$). Then we have

$$\text{(10)} \qquad A\,(t) = [1 - F(t)]\exp\Big\{ - \int\limits_0^t K(\tau)\,d\tau\Big\}\,1(T-t), \qquad t \geqslant 0.$$

In the simple but biologically important case where

$$K(t) \; = \; K_1 1(\theta - t) + K_2 1(t - \theta), \qquad 0 < \theta < T,$$

formulae (10) and (3) imply

$$\text{(11)} \qquad \text{E}\,[n(t)]$$
$$= N\,\frac{\exp\{K_1(T-t)\}-1}{\exp\{K_1 T\}-1}\,[1(\theta - t) + \exp\{(K_1 - K_2)(t - \theta)\}1(t - \theta)], \qquad t \geqslant 0,$$

which agrees with the formula used in [7].

If in the blood there exist two independent red cell subpopulations, differing in values of T or/and K, all the considerations above should be only slightly modified. We omit the details, showing the final formulae for two biologically important cases.

(a) $T_1 \neq T_2$ and $K_1 = K_2 = K$ resulting in

$$\text{(12)} \qquad \text{E}\,[n(t)] \; = \; \sum_{i=1}^{2}\Big[N_i\,\frac{\exp\{K(T_i-t)\}-1}{\exp\{KT_i\}-1}\Big]1(T_i - t), \qquad t \geqslant 0,$$

(b) $T_1,\, T_2 \to \infty$ and $K_1 \neq K_2$ resulting in

$$\text{(13)} \qquad \text{E}\,[n(t)] \; = \; \sum_{i=1}^{2} N_i \exp\{-K_i t\}, \qquad t \geqslant 0,$$

where N_i ($i = 1, 2$) denotes the number of cells of the i-th subpopulation at $t = 0$, and K_i, T_i are the values of the coefficients of random destruction and potential life span, respectively.

The dispersion ϱ satisfies the inequality

$$\varrho(t) \leqslant \frac{1}{\sqrt{\text{E}\,[n(t)]}}.$$

It is interesting that (13) was primarily used as an interpolation formula [4] and later interpreted in terms of two subpopulations [11].

3. Practical implications and computational results. Taking samples of blood containing labelled cells from the patient and measuring the radioactivity we want to know what kind of survival pattern fits the data in the closest way and what are the values of K and T. The information may be of importance for the right diagnosis [7]. The usually obtained empirical values $\tilde{n}(t_j)$ of the cell count at moments t_j are biased with the Gaussian error resulting from superposition of errors of measurements of biological parameters $x_i(t_j)$, necessary to compute $\tilde{n}(t_j)$:

$$(14) \qquad \tilde{n}(t_j) = \tilde{n}[x(t_j)],$$

$$(15) \qquad \delta_j = \Delta\tilde{n}(t_j) = \left\{\sum_i \left[\frac{\partial\tilde{n}}{\partial x_i}\,\Delta x_i(t_j)\right]^2\right\}^{1/2}.$$

The exact forms of (14) and (15) are described e.g. in [5] and [7] and vary according to the technique used by the investigator. But we should always take into account the second error, namely the one resulting from the fact that one tries to fit the realization of a stochastic process to its expected value. It is not a Gaussian error (in our case — binomial) but for very large N it can be similarly approximated. The variance of the total "nearly Gaussian" error is

$$\sigma_j^2 = \mathbf{D}^2[n(t_j)] + \delta_j^2,$$

and the statistics

$$(16) \qquad \Phi = \sum_{j=1}^{j_{max}} \{\mathbf{E}[n(t_j)] - \tilde{n}(t_j)\}^2\,\sigma_j^{-2}$$

has the central χ^2-distribution with j_{max} degrees of freedom and can serve as a tool to compare various fits. If $\mathbf{D}^2(n)$ is comparable with δ_j^2, neglecting $\mathbf{D}^2(n)$ may result in false conclusions. The value of ϱ is, however, a decreasing function of N (see (5)). In the cases which are presented in the sequel, N was estimated in a simple way to be about $2 \cdot 10^8$ (100 ml of blood were labelled with productivity of about 70%, then dissolved in about 4 l of circulating blood, the sample taken contained 3 ml of blood in each t_j, 1 mm^3 of blood contained about $4.5 \cdot 10^6$ red cells). Then (after calculation with such data) $\mathbf{D}^2(n)$ could be neglected.

Data fitting was performed with the aid of a FORTRAN program, minimizing (16) for 8 types of survival patterns. This program, written especially for non-professional users, together with remarks is described in [5]. Details concerning materials and methods used to obtain the experimental data are given in other papers (see [7] and [11]).
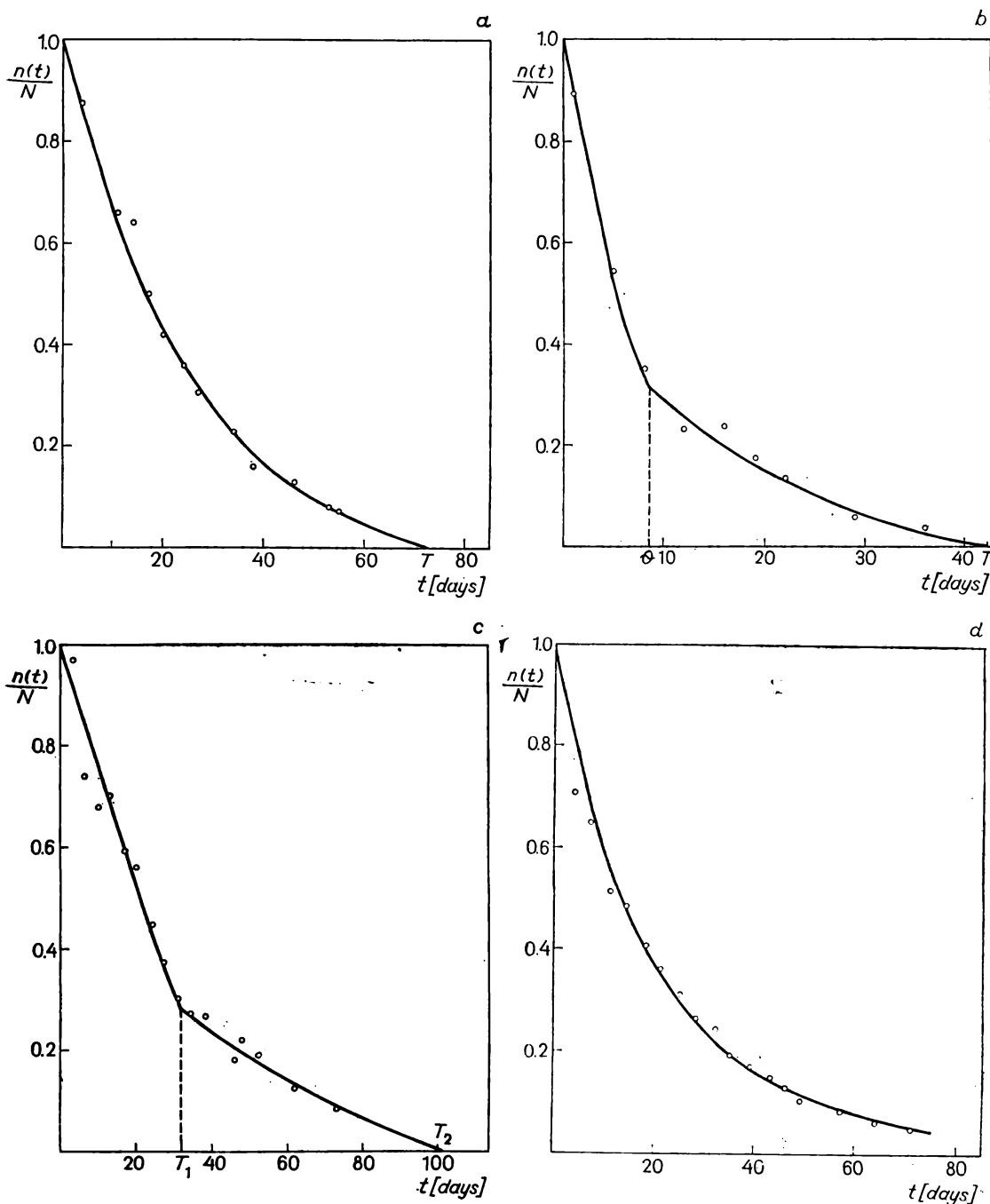
Fig. 2. Empirical data fitted to theoretical survival patterns

a — patient I. K., curve (9), $K = 0.038$, $T = 72.72$, $\Phi = 16.31$, $j_{max} = 12$, $a = 0.85$

b — patient T. M., curve (11), $K_1 = 0.127$, $K_2 = 0.065$, $T = 42.35$, $\theta = 8.68$, $\Phi = 19.38$, $j_{max} = 10$, $a = 0.96$

c — patient I. T., curve (12), $K = 0.01$, $T_1 = 31.98$, $T_2 = 100.53$, $N_2/N = 0.495$, $\Phi = 18.47$, $j_{max} = 16$, $a = 0.7$

d — patient W. K., curve (13), $K_1 = 0.092$, $K_2 = 0.034$, $N_1/N = 0.407$, $\Phi = 15.67$, $j_{max} = 17$, $a = 0.4$

Dimensions: T [days] and K [days^{-1}]. a denotes the probability with which a random variable having χ^2-distribution with j_{max} degrees of freedom is less than Φ and is a standardized measure of risk that the fit is not correct

Figure 2 shows 4 cases of red cell destruction patterns. Case d is of particular interest, since data of patient W. K. could be fitted better ($\Phi = 5.98$) with survival pattern (11) but, for some biological reasons, pattern (13) was the only possible to be used (see [11]). Comparatively high values of Φ in some cases from Fig. 2 and in some cases not considered in this paper might have been caused by:

1. measurement errors greater than usually,
2. the nature of the process differing from our simple model,
3. incorrect estimation of N.

4. Conclusions. The model proposed in the paper was thought as the first approach to the complex stochastic process of erythrocyte production and destruction. The expected values $E[n(t)]$ obtained are similar to those calculated by authors who used averaged quantities. Moreover, estimates for dispersion of the process are obtained, indicating that, in case of small initial red cell count N, the dispersion should be added to the measurement error. In our case it was safely neglected. There are some signals (e.g. unsatisfactory fits mentioned in Section 3) that the real process may be more complicated than the model is. One of the reasons of dispersion greater than our model predicts could be the random character of the parameter T (erythrocyte life span). Another difficult problem is the elution of the radioisotope, changing the shape of survival curve [7]. Investigation of these problems requires more empirical data and new mathematical models.

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STOCHASTYCZNE PODEJŚCIE
DO PROCESU DESTRUKCJI CZERWONYCH CIAŁEK KRWI

STRESZCZENIE

W pracy zaproponowano model zjawiska destrukcji erytrocytów człowieka, określony za pomocą prostego procesu Markowa. Uzyskane teoretyczne krzywe rozpadu porównano z krzywymi otrzymanymi różnymi metodami przez różnych autorów. Rozpatrzono wpływ rozproszenia procesu na dokładność estymacji parametrów populacji erytrocytów.