MATHEMATICAL MODEL OF DEATH AND REPRODUCTION OF MITOCHONDRIA IN THE IRRADIATED ORGANISM

V. V. Sirotkin and S. I. Shchukin

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The traditional method of morphological investigation, which is based on description of the structure of structures and their systematization, is no longer effective because of the widespread introduction of technical and biophysical facilities for the study of biological objects and the need for continuous analysis and generalization of a large volume of data obtained at both cellular and ultrastructural levels. By the use of descriptive methods alone it is impossible to determine the limits of possible errors or to evaluate the role of individual factors in the general development of the process. Consequently, mathematical analysis of the experimental data is an urgent task in biological research. The use of morphometry and construction of mathematical models thus enables not only the generalization of an abundance of experimental data, but also the attainment of qualitatively new information about the object studied.

The aim of this investigation was to create a mathematical model of the processes of death and reproduction of mitochondria of mouse liver cells after whole-body irradiation, on the basis of morphometric parameters.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male CBA × C57BL mice of the same age and weight (22 g). The animals were exposed to γ-irradiation in a dose of 7.5 Gy and a dose rate of 641 rad/min. Respiration of the mitochondria was studied by a polarographic method [2]. Mitochondria were isolated from the liver of every group of 10 animals (five intact and five irradiated mice) on two centrifuges. Time during the series of experiments was counted 5, 30, and 60 min, and thereafter every 60 min until 5 h, after irradiation. To construct the mathematical model data from 14 random samples of areas of isolated mitochondria were used. Each sample consisted of measurement of 150 mitochondria. For the morphometric study the method described previously [4] was used, in which the mitochondria were distributed among classes depending on their area. In the seven control series of experiments six classes were distinguished, in the seven series with irradiation, nine classes were distinguished, because irradiation led to an increase in size of the mitochondria.

EXPERIMENTAL RESULTS

Determination of correlation between changes in size of the mitochondria and their functional activity showed that an increase in size of the large forms of mitochondria was accompanied by depression of their functional activity [2, 4]. According to these parameters, the main functional load falls on mitochondria belonging to class II for size. Testing the homogeneity of the random samples of mitochondria showed that in all series of experiments with irradiation there were significant differences from the control series in the distribution of mitochondria by size. The results of a statistical study and also testing the goodness of fit of the empirical distribution with the theoretical (normal, log-normal, and gamma laws) by Pearson's and Kolmogorov's tests showed that in all the control series of experiments the empirical distributions agree with the gamma-law of distribution. Moreover, no significant differences were observed compared with the gamma-law of distribution in the Kolmogorov test (critical

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probability not below 0.99). In the irradiated animals, on the other hand, a redistribution of the dimensions of the mitochondria was observed, although in this case also the empirical distributions were best approximated by the gamma-law of distribution [1]. The same relationship was established not only for distributions of areas of the isolated hepatocyte mitochondria, but also for mitochondria in cardiomyocytes [6].

The accumulated experience suggests a method of evaluating morphological and functional properties of mitochondria in a population by the use of morphometric criteria [5].

On the basis of analysis of the experimental data we suggested a mathematical model of the change in number of the functionality most active mitochondria under the influence of irradiation.

When constructing the mathematical model of distribution of numbers of mitochondrial populations within each class, the following assumptions, essential in our view, were made. As a result of irradiation the number of the population of the i-th class changes as a result of transitions from the $(i - 1)$-th into the i-th and from the i-th into the $(i + 1)$-th class, and also account of death of the mitochondria. This process can be represented by a kinetic scheme of the first order, characterizing the possibility of mutual transitions between mitochondria of different functional types in the population.

$$\begin{tikzcd}
N_{i-1} \arrow[r, shift left, "K_{i-1}"] \arrow[d] & N_i \arrow[l, shift left, "K_{-(i-1)}"] \arrow[r, shift left, "K_i"] \arrow[d] & N_{i+1} \arrow[l, shift left, "-K_i"] \arrow[d] \\
\mathrm{Kd}_{i-1} & \underset{\text{death}}{\mathrm{Kd}_i} & \mathrm{Kd}_{i+1}
\end{tikzcd}$$

where K_i and K_{-i} are constants of the forward and reverse processes of a change in number, and K_{d_i} the death factor. In accordance with these concepts the change in the number of a population of the i-th class with time assumes the following form:

$$\dot{N}_i = K_{i-1}N_{i-1} + K_{-i}N_{i+1} - K_{-(i-1)}N_i \_ K_iN_i - KrN_i.$$

Thus in order to analyze the number of the population we have a system of differential equations, in which the coefficient K_i and K_{r_i} depend inter alia on the dose of irradiation ΔΦ. This approach, however, requires experimental evaluation of a large number of kinetic constants, so that its practical value is limited.

In this paper we examine a mathematical model of the change in number of the most active class of mitochondria during the development of radiation damage. Analysis of the experimental data shows that it is permissible to examine the change in number of a mitochondrial population which is the result of two global processes: 1) a decrease in the number of mitochondria in constant time τ_1, 2) their reproduction on account of maturation of younger organelles. Under these circumstances it can be accepted that the velocity of the latter process is determined by the deviation N_t from a certain standard value for the population N_{st} which, in turn, depends on the dose of irradiation ΔΦ, the power of the population, the constants of the kinetic processes of maturation and translation of mitochondria belonging to a given class into higher classes. In that case the change in the number N_t at times which are small compared with those characteristic of repair, can be described by the dependence:

$$\frac{dN}{dt} = -\frac{N}{\tau_1} - \frac{N_{st} - N}{\tau_2}, \tag{1}$$

where τ_2 is the characteristic reproduction time of the functionally most active class of mitochondria. The solution to (1) satisfying the condition of equality $N_t|_{t=0}$, where N_0 is the initial value of the number of mitochondria to the condition $t = \infty$

$$\lim_{t \to \infty} N_t = N_{st},$$

has the form

$$N_t = N^0_{\exp}\left\{-\frac{t}{\tau_1}\right\} + N_{st}\left(1 - \exp\left\{-\frac{t}{\tau_2}\right\}\right). \tag{2}$$

The parameters of the model were identified on the basis of the results of the experimental investigations. In the case of short times, allowing for the inequality $\tau_1 << \tau_2$, we have approximately

$$N_t \simeq N^0_{\exp}\left\{-\frac{t}{\tau_1}\right\},$$

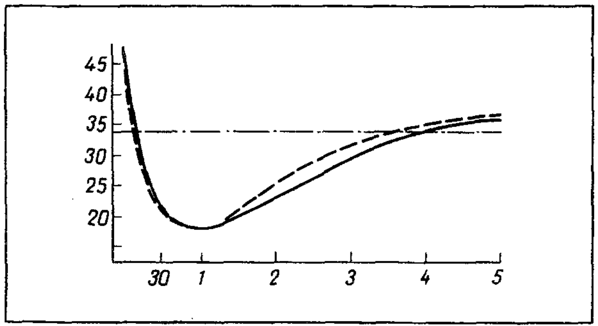

Fig. 1. Dynamics of change in number of functionally active class of liver mitochondria after a single exposure to whole-body ionizing radiation. Continuous line — experimental data (irradiation), broken line — data calculated by model, line of dots and dashes control (without irradiation). Abscissa, time after irradiation; ordinate, number of mitochondria of functionally active class, in %.

whence

$$\tau_1 = -\left[\frac{d\left(\ln\frac{N}{N^0}\right)}{dt}\right]^{-1}$$

i.e., by constructing the graph of the dependence $\ln(N/N^0) = f(t)$ and distinguishing on it the linear portion, we can determine τ_1 from the tangent of the angle of slope. At time $t \sim \tau_2$, and disregarding the first term (see equation 1), by comparison with the second we have:

$$N_t \simeq N_{st} - N_{st}\exp\left\{-\frac{t}{\tau_2}\right\},$$

whence, allowing for the fact that

$$N_{st} = \frac{N}{1-\exp\left\{-\frac{t}{\tau_2}\right\}},$$

we obtain

$$\frac{dN}{dt} = \frac{N_{st} l^{-\frac{t}{\tau_2}}}{\tau_2} = \frac{N_t l^{-\frac{t}{\tau_2}}}{\tau_2\left(1-\exp\left\{\frac{t}{\tau_2}\right\}\right)}$$

or

$$\ln\left[\frac{\frac{dN}{dt}}{N}\right] = \ln\frac{1}{\tau_2} - \frac{t}{\tau_2}.$$

By plotting the graph of dependence of $[(\Delta N/\Delta t)/N]$ on time, we find τ_2 from the tangent of its slope.

The parameters N_{st}, t_1, t_2 were used as an initial approximation during optimization calculations using the suggested model and experimental data. Optimization for three parameters was undertaken on the DVK-2m microcomputer, using gradient methods. The criterion of optimization was the sum of the squares of errors of the model values for the population during time $3\tau_2$ and the experimental data.

Identification of the parameters τ_1 and τ_2 according to the suggested algorithms showed that $\tau_1 = 18 \pm 5$ min and $\tau_2 = 96 \pm 12$ min. Comparison of the data calculated by equation (2) with the experimental data by computer, using Pearson's goodness of fit test showed that the suggested model does not contradict the experimental data with a probability of over 80% (Fig. 1).

Thus, by analogy with [3, 4], we accept that the stationary level to which the number of the population N_{st} tends, depends on certain quasiequilibrium processes, with small doses of irradiation, within the limits of "adaptation" of this quasi-equilibrium process, we can use the equation

$$\ln N_{st} \sim K\Delta\Phi \quad \text{or} \quad \ln \frac{N_{st}(\Delta\Phi)}{N_{st(o)}} = K\Delta\Phi + \text{const},$$

where $N_{st}(\Delta\Phi)$ denotes the level to which the population tends after absorbing energy $\Delta\Phi$, and $N_{st(o)}$ indicates the number of the population in the absence of irradiation.

The experimental data on dependence of the number of the population on dose at long times after irradiation show that these functions are valid and can constitute the basis for more detailed models of the dynamics of the mitochondrial population. The suggested model for a functionally active class of mitochondria is characterized by a high degree of agreement with the experimental results, and for that reason similar assumptions can be used with advantage for the numerical assessment of data in morphological and functional studies of cell organelles and, in particular, during determination of the effect of either pathological or of other damaging factors on the mitochondrial population.

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LIPOFUSCIN DETECTION IN HYBRIDOMA CELL CULTURES

A. B. Tatariunas, V. I. Popov, Yu. A. Mantsygin,
and V. N. Karnaukhov

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It was shown a long time ago that lipid-rich cytoplasmic inclusions, known as lipofuscin granules (LG), accumulate in many postmitotic cells of various mammalian organs and tissues with age, and that they are indeed a characteristic sign of aging [5, 7, 8, 12]. The function of these granules remains a topic for debate [3-5, 7, 8, 12]. Elucidation of the mechanisms of formation and accumulation of LG and their functional role in the cells remains an important task not only of modern gerontology but also of cell biology.

Cell cultures from various tissues and organs provide convenient model systems for research in gerontology [6, 7, 9, 10, 13-15]. Various organotypical [6, 13, 14] and cell cultures [6, 9, 10, 15] have been used for this purpose.

The aim of this investigation was to show that hybridomas constitute a very convenient model with which to study LG formation and accumulation, for they are natural producers of various monoclonal antibodies and they occupy an important place in medico-biological research and modern biotechnology.

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