METHODS

Multiple Correlation Analysis of the Redistribution of Mitochondrial Size in Dependence on the Dose and Time after One-Trial Ionizing Radiation

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In our previous studies we performed multiple correlation analysis to assess the state of the mitochondrial population and the impartiality of the experimental results. It was shown that there is quite a close correlation between the redistribution of the sizes of mitochondria and their morphofunctional types. It was concluded that knowing the area of mitochondria we can reliably determine their functional properties and, conversely, knowing the functional properties, we can judge the area using regression lines [2]. Three-factor correlation analysis of mitochondrial morphofunctional changes was performed according to the results of morphological and biochemical assays and revealed an intimate correlation between the rate respiration of mitochondria , their sizes, and the time after the beginning of the experiment. It was demonstrated that the respiratory rate decreases as the mitochondrial area and time of experiment increased. Furthermore, the larger mitochondria are in size, the shorter their life [3].

The aim of the present investigation was to characterize the state of the mitochondrial population in dependence on various doses and time intervals after irradiation using multifactor correlation analysis.

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MATERIALS AND METHODS.

The experimental method is completely described in our paper devoted to the effect of ionizing radiation on rat cardiomyocyte mitochondrial sizes and attesting to the possibility of approximating the mitochondrial area redistribution by the gamma law of distribution [1]. The experimental data on cardiomyocyte mitochondrial areas at different periods (2, 24, and 120 h after ionizing radiation treatment in different doses: 6.9 and 20 Gy) were used. The data are tabulated in two correlation tables, in which the values of area conform to a group mean of the area of mitochondria (S_i) divided into 9 classes (5, 15, 25, ...85 mm^2 are the group means for the class intervals, measured by electron-microscopy ×10,000).

RESULTS

All experimental data used in the correlation analysis are presented in Tables 1 and 2. The specificity of the data offers the possibility of obtaining a general picture of regression, as in the correlation analy-

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TABLE 1. Correlation Analysis

t_i, h	N_i, Gy	$\overline{S}_i$, mm²	n_i
2	6	23.04	138
24	9	17.53	150
120	20	15.8	150
Total			438

sis, for example, each value of t requires a definite value of N, i.e., as the time t changes, so does the dose of radiation N .

In Table 1 , for example, the first row means that after two hours of 6 Gy radiation, 138 mitochondria with a group mean are of 23.04 mm² were revealed.

An equation characterizing the dependence of mitochondrial area on radiation dose and time after treatment has the following general aspect:

$$S = \overline{S} + a \cdot (t - \overline{t}) + b \cdot (N - \overline{N}).$$

Parameters a and b are:

$$a = \frac{r_{tS} - r_{tN} \cdot r_{NS}}{1 - r_{tN}^2} \cdot \frac{\sigma_S}{\sigma_T};$$

$$b = \frac{r_{NS} - r_{tS} \cdot r_{tN}}{1 - r_{tN}^2} \cdot \frac{\sigma_S}{\sigma_N};$$

σ_t, σ_N, and σ_S are the common mean square deviations of variables t, N, and S, respectively. They are:

$$\sigma_t = \sqrt{\frac{\sum_{i=1}^{3} (t_i - \overline{t})^2 \cdot n_i}{n}};$$

$$\sigma_N = \sqrt{\frac{\sum_{i=1}^{3} (N_i - \overline{N})^2 \cdot n_i}{n}};$$

$$\sigma_S = \sqrt{\frac{\sum_{i=1}^{3} (\overline{S}_i - \overline{S})^2 \cdot n_i}{n}};$$

r_{tN}, r_{tN}, and r_{NS} are the correlation coefficients between the corresponding pairs of variables. They are:

$$r_{tN} = \frac{\overline{tN} - \overline{t} \cdot \overline{N}}{\sigma_t \cdot \sigma_N}; \quad r_{tS} = \frac{\overline{tS} - \overline{t} \cdot \overline{S}}{\sigma_t \cdot \sigma_S}; \quad r_{NS} = \frac{\overline{NS} - \overline{N} \cdot \overline{S}}{\sigma_N \cdot \sigma_S}.$$

TABLE 2. Correlation Analysis

t_i, h	N_i, Gy	$\overline{S}_i$, mm²	n_i
2	20	22	200
24	9	17.53	150
120	6	17.8	150
Total	–	–	500

In all these formulas t, N, S, tN, tS, NS are the means of variables t, N, *and* S, and their paired products.

$$\overline{t} = \frac{\sum_{i=1}^{3} t_i \cdot n_i}{n}; \quad \overline{N} = \frac{\sum_{i=1}^{3} N_i \cdot n_i}{n}; \quad \overline{S} = \frac{\sum_{i=1}^{3} \overline{S}_i \cdot n_i}{n};$$

$$\overline{tN} = \frac{\sum_{i=1}^{3} t_i \cdot N_i \cdot n_i}{n}; \quad \overline{NS} = \frac{\sum_{i=1}^{3} N_i \cdot S_i \cdot n_i}{n};$$

$$\overline{tS} = \frac{\sum_{i=1}^{3} t_i \cdot S_i \cdot n_i}{n}.$$

Let us make the necessary calculations:

$$\overline{t} = \frac{2 \cdot 138 + 24 \cdot 150 + 120 \cdot 150}{438} = 46.95 \text{ (h)};$$

$$\overline{N} = \frac{6 \cdot 138 + 438 \cdot 150 + 20 \cdot 150}{438} = 11.82 \text{ (Gy)};$$

$$\overline{S} = \frac{23.04 \cdot 138 + 17.53 \cdot 150 + 15.8 \cdot 150}{438} = 18.67 \text{ (mm}^2\text{)};$$

$$\overline{tN} = \frac{2 \cdot 6 \cdot 138 + 24 \cdot 9 \cdot 150 + 120 \cdot 20 \cdot 150}{438} = 899.67;$$

$$\overline{NS} = \frac{6 \cdot 23.04 \cdot 138 + 9 \cdot 17.53 \cdot 150 + 20 \cdot 15.8 \cdot 150}{438} =$$

$$= 205.81;$$

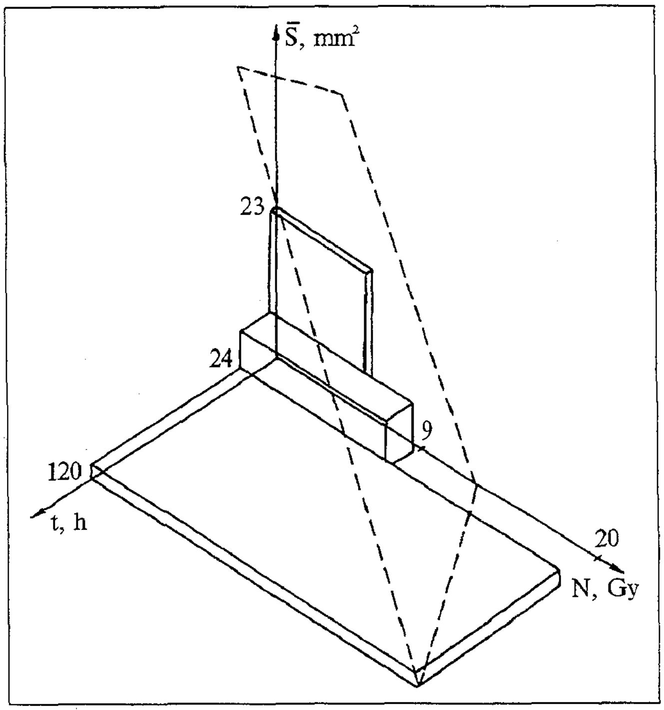

Fig. 1. Plot of the empirical data and regression plane of time, area group mean, and radiation dose (Table. 1).

$$\overline{tS} = \frac{2 \cdot 23.04 \cdot 138 + 24 \cdot 17.53 \cdot 150 + 120 \cdot 15.8 \cdot 150}{438} =$$

$= 807.92.$

Now let us find the standard deviations:

$$\sigma_t = [\frac{(2-49.95)^2 \cdot 138 + (24-49.95)^2 \cdot 150 +}{438} \rightarrow$$

$$\rightarrow \frac{+(120-49.95)^2 \cdot 150}{}]^{\frac{1}{2}} = 51.34;$$

$$\sigma_N = [\frac{(6-11.82)^2 \cdot 138 + (9-11.82)^2 \cdot 150 +}{438} \rightarrow$$

$$\rightarrow \frac{+(20-11.82)^2 \cdot 150}{}]^{\frac{1}{2}} = 6.03;$$

$$\sigma_S = [\frac{(23.04-18.67)^2 \cdot 138 + (17.53-18.67)^2 \cdot 150 +}{438} \rightarrow$$

$$\rightarrow \frac{+(15.8-18.67)^2 \cdot 150}{}]^{\frac{1}{2}} = 3.05.$$

Substituting the values obtained into the formulas, we can calculate the correlation coefficients:

$$r_{tN} = \frac{899.67 - 49.95 \cdot 11.82}{51.34 \cdot 6.03} = 0.999;$$

$$r_{tS} = \frac{807.92 - 49.95 \cdot 18.67}{51.34 \cdot 3.05} = 0.796;$$

$$r_{NS} = \frac{205.81 - 11.82 \cdot 18.67}{6.03 \cdot 2.05} = -0.808.$$

Then the equation coeffieients a and b are:

$$a = \frac{-0.796 - 0.999 \cdot (-0.808)}{1-(0.999)^2} \cdot \frac{3.05}{51.34} = 0.333;$$

$$b = \frac{(-0.808) - (-0.796) \cdot 0.999}{1-(0.999)^2} \cdot \frac{3.05}{6.03} = -3.238.$$

The equation is written as follows:

$$\overline{S}_1 = 18.67 + 0.333 \cdot (t - 49.95) - 3.238 \cdot (N - 11.82);$$

$$\overline{S}_1 = 18.67 + 0.333 \cdot t - 16.63 - 3.238 \cdot N + 38.27.$$

In its final form,

$$\overline{S}_1 = 0.333 \cdot t - 3.238 \cdot N + 40.31.$$

Thus, we found an equation of the linear correlation between three variables. This plane is depicted on Figure 1 (the boundaries are the dotted lines). Figure 1 also presents the experimental data as a histogram.

Let us determine the closeness of the linear correlation between the variables. For this purpose, the combined correlation coefficient is to be calculated:

$$R = \sqrt{\frac{r_{tS}^2 + r_{NS}^2 - 2 \cdot r_{tS} \cdot r_{NS} \cdot r_{tN}}{1 - r_{tN}^2}}.$$

In this case: R=0.85.

Analyzing the obtained values of the correlation coefficients, it can be assumed that: 1) S_i can be described by linear correlations with time and area. The correlation coefficient is close to 1 and R reflects the closeness of the linear connection of S with t and N, and the closer R is to 1, the greater the closeness of the linear correlation of S with t and N. Thus, knowing the radiation dose and the time after irradiation, we can reliably determine the value of S_i using the equation $S=f(t,N)$; 2) the negative coefficient before N in the equation indicates a decrease of S with an increase of the radiation dose. In other words, an increase of the radiation dose in a fixed time period leads to an increase in the mortality of the large, "swollen" mitochondria. The mitochondria of the preceding classes (smaller mitochondria of the intermediate type) are drawn into this process.

Correlation analysis of the following data (Table 2) is performed to get a fuller picture of the regression analysis.

All the mathematical operations are the same as before, and so we present just the results of the calculations.

$\overline{t}$=44 (h); $\overline{N}$=12.5 (Gy); $\overline{S}$=19.399 (mm²);

$$\overline{tN} = 296.8; \quad \overline{NS} = 255.371; \quad \overline{tS} = 784.616;$$

$$\sigma_t = 50.58; \quad \sigma_N = 6.23; \quad \sigma_S = 2.126;$$

$$r_{tN} = \frac{296.8 - 44 \cdot 12.5}{50.58 \cdot 6.23} = -0.804;$$

$$r_{tS} = \frac{784.616 - 44 \cdot 19.399}{50.58 \cdot 2.126} = -0.641;$$

$$r_{NS} = \frac{255.371 - 12.5 \cdot 19.399}{6.23 \cdot 2.126} = 0.973;$$

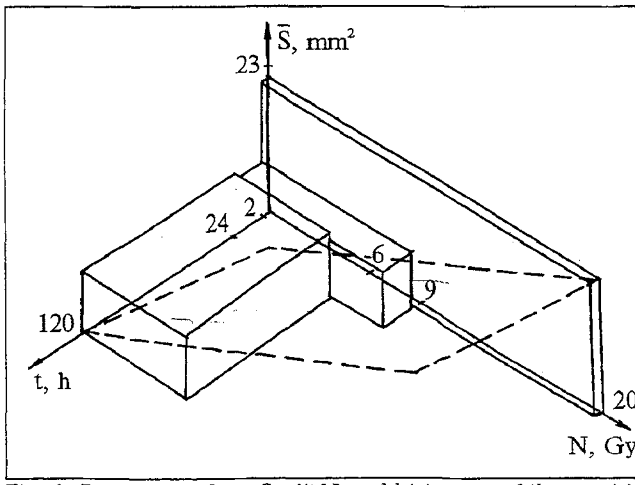

Fig. 2. Regression plane S_2=$f(t,N)$ and histogram of the empirical data (Table. 2).

$$a = \frac{-0.641 - (-0.804) \cdot 0.973}{1 - (-0.804)^2} \cdot \frac{2.126}{50.58} = 0.017;$$

$$b = \frac{0.973 - (-0.641) \cdot (-0.804)}{1 - (-0.804)^2} \cdot \frac{2.126}{6.23} = 0.442.$$

The equation is written as follows:

$$\overline{S}_2 = 19.399 + 0.017 \cdot (t - 44) + 0.442 \cdot (N - 12.5);$$

$$\overline{S}_2 = 19.399 + 0.017 \cdot t - 0.748 + 0.442 \cdot N - 5.525;$$

$$\overline{S}_2 = 0.017 \cdot t + 0.442 \cdot N + 13.126.$$

Thus, we found a one more equation of three variables. This plane is depicted on Fig. 2 by a dotted line.

Let us determine the closeness of the linear correlation between the variables. The combined correlation coefficient is: R=1.00(15933).

The influence of t on S for fixed N and N on S for fixed t will be assessed using the specific correlation coefficients, respectively:

$$r_{t\overline{S}(N)} = \frac{(-0.641) - (-0.804) \cdot (0.973)}{\sqrt{1 - (-0.804)^2 \cdot (1 - (-0.641)^2}} =$$

$$= \frac{0.141292}{0.137243} = 1.0(294988);$$

$$r_{N\overline{S}(t)} = \frac{0.973 - (-0.804) \cdot (-0.641)}{\sqrt{1 - (-0.804)^2 \cdot (1 - (-0.641)^2}} =$$

$$= \frac{0.457636}{0.4564022} = 1.00(27031).$$

Thus, we obtained: R=1;

$$r_{t\overline{S}(N)} = 1; \; r_{N\overline{S}(t)} = 1.$$

Thus, the group mean of the area of the population of the irradiated mitochondria may be described by the linear correlation $\overline{S}=f(t,N)$.

The positive coefficients a and b in the linear regression equation indicate the elevation of $\overline{S}$ with an increase of N and t. Analysis of the specific correlation coefficients $r_{t\overline{S}(N)}$ and $r_{N\overline{S}(t)}$ reveals that, since they are equal to 1, we can reliably determine $\overline{S}$ as a function of t (N is fixed) and of N (t is fixed) using a regression equation.

Thus, it may be concluded from our own experimental findings and published data that multifactor correlation analysis, along with descriptive morphology and morphometry, is becoming a necessary tool in medical and biological research for an objective interpretation of the empirical data.

REFERENCES

1. V. V. Sirotkin, G. G.Avtandilov, and N. D. Volodin, *Byull., Eksp. Biol.*, **106**, № 12, 751-754 (1988).
2. V. V. Sirotkin, A. Yu. Grishin, V. A. Smirnov, *et al.*, *Ibid.*, **115**, № 2, 215-218 (1993).
3. V. V. Sirotkin, A. Yu. Grishin, V. A. Smirnov, *et al.*, *Ibid.*, **115**, № 3, 303-305 (1993).