Two-factor Correlation Analysis of the Distribution of Morphofunctional Properties of Mitochondria during Irradiation

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It is a moot point of cellular biology how to objectively assess functional variations of the cell organelles, including mitochondria, according to their ultrastructural image [4,5].

Scientists usually apply qualitative criteria in electron microscopic studies of cell organelles. However, the prospects for the morphometric method of analyzing ultrastructural features of cells and tissues have been demonstrated in several series of investigations [1].

During the last ten years, many investigators have been seeking to develop automated systems for cytological studies, and it has become necessary to devise an electron microscopic technique to improve such an approach. In this connection the definition of morphometric criteria for an appraisal of the morphofunctional properties of organelles and the establishment of mathematical algorithms are modern goals of medicine and and biology for the furtherment of our knowledge in cell biology.

The aim of this investigation was to establish the correlation between the known morphofunctional types of mitochondria and the redistribution of their areas under normal and radiation-treatment conditions.

MATERIALS AND METHODS

Experiments were carried out on male albino rats weighing 150-200 g, exposed to local ionizing radiation in a dose of 20 Gy in the heart region. Specimens from the subendocardial layer of the left ventricle lateral wall were dissected. The portions of myocardium of both the control and treated animals were fixed with glutaraldehyde by the routine method and embedded in Epon-Araldite. Ultrathin sections were studied under a JBM-100B microscope at 10,000 power. The test objects were 1500 mitochondria of intact and irradiated rats. The morphometry of the mitochondria was performed from the screen of the electron microscope using our adaptation [10,12].

The values of mitochondrial areas were analyzed using continuous variation series and according to A.Sterdges formula:

$$i = \frac{x_{max} - x_{min}}{1 + 3.32 \ln n},$$

where the minimal and maximal values were taken into account and according to the obtained results

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separated into classes. We obtained six classes of mitochondria for the control animals and nine classes for the treated animals, due to the increased sizes of the mitochondria caused by the radiation procedure. Each sample consisted of 150 tests. Ten experimental series were performed: 3 control series and 7 series using ionizing radiation.

RESULTS

Qualitative ultrastructural analysis showed the impossibility of classifing the observed mitochondria according to known morphofunctional types. Marked destruction of mitochondria manifested in an increase of their areas, disintegration of the internal and external membranes and also in the formation of intramitochondrial vacuoles, results from irradiation. Some mitochondria, however, preserve their ultrastructure. Myofibril lysis and mitochondria with an electron-dense matrix (so-called condensed form of organization) are noted.

Such a mosaic structure of mitochondria of the same cells is related to the law of intermittent activity of cell organelles. At one and the same time the organelles express different levels of functional activity. Active mitochondria are subject to more profound structural changes under treatment than those with less activity [7]. The interrelation between these populations of organelles and their transition from one morphofunctional type and another is described by a first-order kinetic scheme [11].

Chance's correlation between four metabolic states and ultrastructural alterations of mitochondria [14] has lent impetus to research among morphologists into the structural and functional changes of these cell organelles. However, the findings to date are estremely contradictory.

The use of morphometric and mathematical analysis of the ultrastructural image of cell organelles, and of mitochondria in particular, could serve to compare numerous existing experimental data. The necessity of such an approach was formulated by N. K. Monakhov. He proposed that the quantitative relations between different classes of functionally active mitochondria could be taken into account to assess their entire population [6]. However, this division according to known morphofunctional types is not always possible because, using ultrastructural analysis, one could obtain at best two main types of mitochondria with dense and translucent matrix and a multitude of intermediate forms [13]. That is why the grading of mitochondria in such a way is very difficult and often subjective.

In previous work we studied the functional state of hepatocyte mitochondria using the basic morphological test of the area. We obtained a specific correlation between the redistribution of the areas of the mitochondria and their functional activity [2]. This work presents added proof of the validity of the morphometric test chosen.

Figure 1 depicts the entire body of experimental material obtained by morphometric analysis of cardiomyocyte mitochondria for irradiation of animals

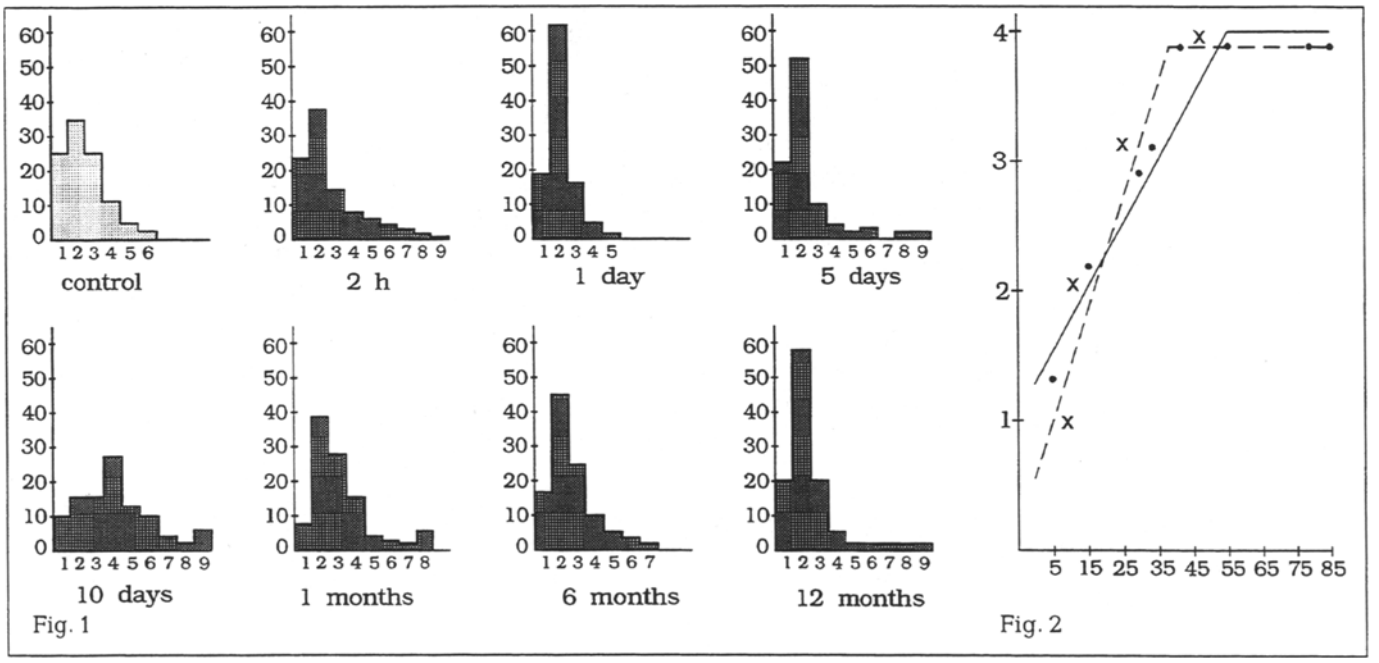

Fig. 1. Size distribution of cardiomyocyte mitochondria at different periods after irradiation, (dose 20 Gy).

Fig. 2. Plots of group means w versus s and s versus w.

with a dose of 20 Gy. The histograms present the experimental series of control and treatment at different time intervals. Whereas the three control series depicted on the first histogram differ insignificantly, all the histograms of the experimental series under irradiation show a redistribution of the mitochondrial areas with significant differences from each other and from the control groups under the Pearson goodness-of-fit test. Statistical analysis showed that the redistributions of areas under normal conditions and with radiation treatment with different doses at different times are approximated by the Gamma-law of distribution [3,8]. These size redistributions are described by a simulated mathematical model of morphofunctional alterations of mitochondria [9].

To perform the correlation analysis between the redistributions of mitochondria sizes and known morphofunctional mitochondria types we used a previously described classification (C.Hackenbroock, 1966).

I. Tighly conjugated, condensed - mitochondria are small, membranes preserved, matrix is electron-opaque.

II. Labilely conjugated, orthodox mitochondria are of usual size, membranes preserved, matrix of moderate electron density.

III. Loosely conjugated, intermediate this type of mitochondria exhibits volume changes usually reported as "swelling" sometimes the membranes are destroyed, matrix of moderate electron density.

IY. Weakly conjugated, intermediate mitochondria swollen cristae are fragmented, membranes often distroyed matrix electron-translucent.

It should be mentioned that the above classification presents no well-defined boundaries between mitochondria types. In more exact terms each type conforms to an interval which embraces mitochondria with similar morphofunctional indicators. From this viewpoint the distribution of mitochondria may sometimes be characterized by a mean group value (hypothetical value of some class in which mitochondria are concentrated).

It should be noted that the above types of mitochondria were not observed in all series of experiments. With the highest reliability we were able to find these morphofunctional types in the experimental series using 20 Gy, five hours after treatment. The results obtained in the control series gave no possibility of such a classification.

The empirical data and calculated group means are tabulated in a unified correlation table (Table 1).

Thus, for example, number 57 situated at the intersection of the second row and the fourth column means that after five hours of 20 Gy radiation, 57 mitochondria with an area of 15 mm^2 and classified as the second structural type were observed. Henceforth the values of the areas are given in mm^2 and the functional types in relative units.

The data cited in the table show that there is a correlation between the areas of the mitochondria and the intervals (mitochondria types) in which they are grouped. It is convenient to estimate the degree of this correlation by the correlation coefficient if the normalized correlation coefficient r is greater than 0.5. As applied to the problem at hand, the normalized correlation coefficient is calculated according to the formula

$$r = \frac{\overline{sw}}{\sqrt{\bar{s}^2 \cdot \bar{w}^2}},$$

where the line at the top signifies the operation of averaging, $\bar{s}^2$ and $\bar{w}^2$ are the mean square value of, respectively, s and w ($\sqrt{\bar{s}^2}$ and $\sqrt{\bar{w}^2}$ characterize the scatter of s and w around averages $\bar{s}$ and $\bar{w}$).

$$\bar{s}^2 = \frac{\sum_{i=1}^{9}\sum_{j=1}^{4} s_i^2 \cdot n_{ij}}{n}; \quad \bar{w}^2 = \frac{\sum_{j=1}^{4}\sum_{i=1}^{9} w_j^2 \cdot n_{ij}}{n}.$$

After calculating we get $\bar{s}^2$=393, $\bar{w}^2$=4.86, and the normalized correlation coefficient will be

TABLE 1.

Classes of mitochondria	w_j / s_i	Morphofunctional types of mitochondria				n_{Si}	w_i
		I	II	III	IV		
1	5	30	13	1	0	44	1.341
2	15	6	57	12	1	76	2.105
3	25	0	5	13	0	18	2.722
4	35	0	0	5	2	7	3.286
5	45	0	0	0	1	1	4
6	55	0	0	2	0	2	3
7	65	0	0	0	0	0	0
8	75	0	0	0	1	1	4
9	85	0	0	0	1	1	4
	n_{wj}	36	75	39	6	150	
	s_j	6.667	12.267	24.1	48.333	0	

$$r = \frac{37.233}{393 \cdot 4.86} = 0.85,$$

which is more than 0.5, so the correlation between mitochondrial areas and morphostructural types is rather strong. To establish the strength and closeness of the relationship between variables w and s, we performed two-factor correlational analysis. The group means, which are the arithmetic means of variables s and w, are the following:

$$\bar{s}_j = \frac{\sum_{i=1}^{9} s_i \cdot n_{ij}}{n_{wi}}, (j = 1,2,3,4),$$

$$\bar{w}_i = \frac{\sum_{j=1}^{4} w_j \cdot n_{ij}}{n_{si}}, (i = 1,2,3...9).$$

So, for $\bar{s}_j$ $\bar{w}_i$: $\bar{s}_i$=6.667; $\bar{w}_i$=1.341

The results of the calculations are shown in Table 1.

It should be noted that variable $\bar{w}_i$ caracterize the group mean of some hypothetical class which contains mitochondria of size s_i.Variable $\bar{s}_j$ is a measure of the middle size of mitochondria existing in the j-th class.

Let us determine the linear relationship between two sequences specified by the aggregate of data s_i and w_j. What will interest us most is the connection between mitochondria size and morphofunctional properties, and vice versa.

On the basis of these data, we can plot the relationship between group means $\bar{w}$ and s and group means $\bar{s}$ and w (Fig. 2). Solid points represent - $\bar{w}_i$, daggers represent - $\bar{s}_j$.

For the next step it is necessary to define the equation of regression lines for w to s:

$$w - \bar{w} = \rho_{w/s}(s - \bar{s})$$

for s to w:

$$s - \bar{s} = \rho_{s/w}(w - \bar{w}).$$

$\rho_{w/s}$ and $\rho_{s/w}$ here are the respective regression coefficients; $\bar{w}$ and $\bar{s}$ are total means for variables s and w.

$$\rho_{w/s} = \frac{\mu}{\sigma_s^2}; \rho_{s/w} = \frac{\mu}{\sigma_w^2}; \mu = \overline{sw} - \bar{s} \cdot \bar{w}.$$

Unknown values may be calculated according to the following formulas:
for total means:

$$\bar{w} = \frac{\sum_{j=1}^{4} w_j \cdot n_{wj}}{n}; \bar{s} = \frac{\sum_{i=1}^{9} s_i \cdot n_{si}}{n};$$

the numerator of the regression coefficients:

$$\mu = \frac{\sum_{i=1}^{9}\sum_{j=1}^{4} s_i \cdot w_j \cdot n_{ij}}{n} - \bar{s} \cdot \bar{w};$$

for the variance of variables w and s relative to their total means:

$$\sigma_w^2 = \sum_{j=1}^{4} (w_j - \bar{w})^2 \cdot n_{wj} / n; \sigma_s^2 = \sum_{i=1}^{9} (s_i - \bar{s})^2 \cdot n_{si} / n.$$

After calculation:

$$\bar{s} = 15.8; \bar{w} = 2.06; \overline{sw} = 39.17;$$

$$\mu = 39.17 - 15.8 \cdot 2.06 = 6.622;$$

$$\sigma_w^2 = 0.16; \sigma_s^2 = 143.36.$$

Thus:

$$\rho_{w/s} = \frac{6.622}{143.36} = 0.05,$$

$$\rho_{s/w} = \frac{6.622}{0.616} = 10.8.$$

Then the equations of regression lines are written as follows: for regression w to s:

$$w = 0.05 \cdot s + 1.27, s > 0.$$

But since morphofunctional type IV is the last of the known mitochondria types a constraint is to be put upon regression line w to s:

$$w = 0.057 \cdot s + 1.27, \text{ for } s \in]0;54[;$$

$$w = 4, \text{ for } s \in [54;85].$$

Figure 1 shows a solid line for the regression s to w:

$$s = 10.8 \cdot w - 6.45, w > 0.$$

Again as the fourth morphofunctional type is the last one,

$$s = 10.8 \cdot w - 6.45, \text{ for } w \in]0;4[;$$

This is a discontinuous line on the graph.

To determine the strength and the closeness of the relationship between variables w and s, let us find the correlation coefficients and ratios.

The correlation coefficient:

$$r = \pm\sqrt{\rho_{w/s} \cdot \rho_{s/w}}.$$

The sign is chosen depending upon the signs of w and s. We got

$$r = \sqrt{0.05 \cdot 10.8} = 0.735.$$

The correlation ratios are:

$$\eta_{ws} = \frac{\delta_w}{\sigma_w}; \eta_{sw} = \frac{\delta_s}{\sigma_s},$$

where δ_w and δ_s are the variances between groups:

$$\delta_w = \sqrt{\frac{\sum_{i=1}^{9} (\bar{w}_i - \bar{w})^2 \cdot n_{si}}{n}};$$

$$\delta_s = \sqrt{\frac{\sum_{j=1}^{4} (\bar{s}_j - \bar{s})^2 \cdot n_{wj}}{n}},$$

σ_w and σ_s are

$$\sigma_w = \sqrt{0.616}, \sigma_s = \sqrt{143.36}.$$

In our case δ_w=0.602; δ_s=9.104; σ_w=0.785; σ_s=11.973. Accordingly, η_{ws}=0.602/0.758=0.78; η_{sw}= =9.104/11.973=0.76. Thus, r=0.7; η_{ws}=0.8; η_{sw}=0.8.

The closer the absolute value of the correlation coefficient r is to 1, the smaller the mean square error of determination of w according to the equation of the w to s regression line and of s according to the equation of the s to w regression line, that is, the closer the correlation coefficient is to +1, the closer the values of w lie to the regression line w to s and the values of s to regression line s to w. It thus may be concluded that the dependence of the functional properties of mitochondria on their area can be described using the equation of the w to s regression line. The greater a mitochondrion's area, the lower is its functional activity, and vice versa, although inactive mitochondria can fall into groups with a small area. The correlations bear this out. Actually, the regressions w to s and s to w are not strictly linear, because

$$\eta_{ws} \neq |r|, \eta_{sw} \neq |r|.$$

Hence, knowing the area of a mitochondrion, we can reliably determine its functional properties and, conversely, knowing the functional properties, we can judge the area using the regression lines w to s and s to w.

This is further justification (alheit open to discussion) for using the previously proposed method of estimating morphofunctional changes of mitochondria in electron microscopic studies [9] and mathematical models based on experimental data characterizing the morphofunctional changes of these cell organelles. We hope that this approach proves useful to morphologists and biochemists in future investigations.

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