EFFECT OF IRRADIATION ON SIZE OF ISOLATED LIVER MITOCHONDRIA

G. G. Avtandilov, V. V. Sirotkin,
Yu. M. Dovzhenko, and A. P. Lobzin

UDC 612.359.014.21.014.482

KEY WORDS: mitochondria; statistical analysis; classes; irradiation; laws of distribution; Pearson and Kolmogorov tests.

Wide gradations in the degree of coupling between respiration and oxidative phosphorylation, which may depend not only on changes in the functional properties of the mitochondrial population as a whole, but also on changes in the distribution of mitochondria of different types [4], are now known. Close correlation was observed previously [2, 3, 5] between the functional activity of mitochondria and their size.

The aim of this investigation was to study changes in hepatocyte mitochondria during exposure to some external factors, as reflected in the dynamics of changes in their size.

EXPERIMENTAL METHOD

Fourteen random samples of dimensions of visible areas of isolated liver mitochondria from intact and irradiated animals were analyzed. Each sample included 150 mitochondria taken from the total mitochondrial fractions isolated from the liver of five hybrid male (CBA × C57BL) mice of the same age and the same weight (22 g). Mitochondria were isolated from each of five organs on two centrifuges (parallel procedures for the control — normal — and after γ-irradiation of the animals in a dose of 750 R with a dose rate of 641 R/min). The time of the experiments was counted after irradiation (5, 30, and 60 min after irradiation, and thereafter every 60 min until the 5th hour of the experiment).

Mitochondria were isolated by differential centrifugation in medium of the following composition: 0.3 M mannitol, 0.01 M Tris-buffer, and 0.1 mM EDTA, pH 7.4. The areas of the mitochondria were selected as the morphometric test for evaluation of their state. Morphometric studies were carried out by the method suggested previously [2, 5], according to which the dimensions of the mitochondria were distributed among classes. Six classes were distinguished in the control experiments, nine classes in the experiments with irradiation, for irradiation led to an increase in size of the mitochondria. To test the hypotheses on the laws of distribution, and also on the distribution of areas of the mitochondria, the RD-1 program [1] was used. The hypothesis of homogeneity was tested by the χ^2 test. The calculations were done on the EVM-1022 computer. Pearson's and Kolmogorov's tests were used mainly to interpret the data, with the addition of sample characteristics of the empirical distribution: the arithmetic mean, standard deviation, and mode.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the arithmetic mean values of the areas of isolated mitochondria under normal conditions are closely similar and vary within a narrow interval: from 17.34 to 19.88. The same values, characterizing arithmetic mean dimensions of mitochondria in irradiated animals were 19.6 in the experiments of series I, 35.54 in those of series II, 23.19 in those of series III, and in each successive experiment 33.53, 17.5, 24.9, and 26.42, respectively. The results thus indicate that mean values of areas of liver mitochondria under normal conditions lie at one limit and form a straight line on the graph. These same values in irradiated animals vary within a wide interval and reach twice the control values. The arithmetic means indicate that in the control experiments these values belong to the 2nd and 3rd classes, whereas in the experiments with irradiation they belong to the 2nd, 3rd, and

Central Research Laboratory and Department of Pathological Anatomy, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, G. N. Kryzhanovskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 409-411, April, 1986. Original article submitted May 21, 1985.

0007-4888/86/0004-0429$12.50

TABLE 1. Sample Characteristics of Empirical Distribution

No. of series of experiments	Time after irradiation	Characteristics of empirical distribution		
		arithmetic mean	standard deviation	Mode B
		Control		
1	5 min	19,88±2,0	12,32	14,65
2	30 min	19,46±2,0	12,58	14,25
3	1 h	19,72±2,0	12,65	14,26
4	2 h	18,33±1,8	11,12	14,18
5	3 h	19,44±1,8	11,25	14,74
6	4 h	17,34±1,9	10,53	13,9
7	5 h	17,41±1,8	10,86	13,56
		Irradiation		
8	5 min	19,6±2,2	13,65	13,38
9	30 min	13,54±2,7	16,86	34,55
10	1 h	23,19±3,2	19,96	1,00
11	2 h	33,52±3,4	20,83	23,71
12	3 h	17,48±2,6	16,45	1,00
13	4 h	24,94±2,7	16,65	16,08
14	5 h	26,42±3,1	19,55	14,67

TABLE 2. Pearson's and Kolmagorov Tests of Goodness to Fit for Three Laws of Distribution

No. of series of experiments	Time after irradiation	Pearson's test of goodness to fit (χ)			Kolmogorov's test for goodness of fit			Critical probability for gamma distribution
		for normal distribution	for lognormal distribution	for gamma distribution	for normal distribution	for lognormal distribution	for gamma distribution	
				Control				
1	5 min	22,79	—	2,63	1,29	0,58	0,29	1
2	30 min	22,11	—	1,88	1,36	0,56	0,32	1
3	1 h	22,11	—	1,88	1,36	0,56	0,32	1
4	2 h	12,58	—	1,1	0,98	0,6	0,22	1
5	3 h	16,16	—	3,28	1,65	0,65	0,41	0,99
6	4 h	11,68	—	0,68	0,92	0,47	0,16	1
7	5 h	14,95	—	1,18	1,05	0,47	0,15	1
				Irradiation				
8	5 min	44,11	—	17,55	2,06	0,97	0,94	0,34
9	30 min	14,42	—	7,78	0,66	0,9	0,58	0,88
10	1 h	87,05	—	13,47	1,63	1,82	1,03	0,24
11	2 h	34,7	—	16,88	1,15	1,03	0,74	0,64
12	3 h	89,84	—	5,9	1,95	0,94	0,53	0,94
13	4 h	24,67	—	9,29	1,26	0,31	0,36	1
14	5 h	55,84	—	14,75	1,9	0,84	0,85	0,47

*The statistics and probability of Pearson's X^2 criterion is not calculated since the number of degrees of freedom is negative

4th classes, depending on the time elapsing after irradiation. Values of the mode in the control experiments indicate that the largest number of mitochondria belong to the second class. In the experiments with irradiation, besides the second class, values of the mode characterizing the largest number of mitochondria belonging to the 1st, 3rd, and 4th classes also appear. These data indicate that the main functional activity is carried out by mitochondria normally belonging to the 2nd and 3rd classes. Changes in functional activity after irradiation can be explained by redistribution of areas of mitochondria caused by irradiation, which is accompanied by a fall in their respiratory activity [2]. Changes observed in the distributions of areas of the mitochondria can be explained, in our opinion, by their powers of adaptation. Under the influence of irradiation the number of mitochondria of large size is increased, and this may be evidence of their functional insufficiency. The increase in the number of small mitochondria can be regarded as a sign of intensification of intracellular regeneration of subcellular structures.

The results of this more penetrating statistical analysis of mitochondria, as well as testing the goodness of fit of the empirical distribution with theoretical distributions (normal, lognormal, and gamma-laws) using Pearson's and Kolmogorov's tests showed that empirical distributions in the control experiments conformed most completely to the gamma-law of

TABLE 3. Parameters of Gamma-Law of Distribution

No. of series of experiments	Time after irradiation	Parameter of gamma-law of distribution	
		scale (a)	scale (b)
Control			
1	5 min	0,13±0,01	2,63±0,26
2	30 min	0,12±0,01	2,41±0,25
3	1 h	0,12±0,01	2,45±0,25
4	2 h	0,15±0,01	2,74±0,26
5	3 h	0,15±0,01	3,00±0,27
6	4 h	0,16±0,01	2,73±0,27
7	5 h	0,15±0,01	2,59±0,25
Irradiation			
8	5 min	0,11±0,01	2,08±0,23
9	30 min	0,13±0,01	4,47±0,3
10	1 h	0,06±0,01	1,36±0,23
11	2 h	0,08±0,01	2,6±0,25
12	3 h	0,06±0,01	1,14±0,17
13	4 h	0,09±0,01	2,26±0,24
14	5 h	0,07±0,01	1,84±0,22

distribution. This is clear from the values of χ^2 and λ given in Table 2, which were obtained by comparing empirical data with the normal, lognormal, and gamma-laws of distributions; no significant differences, moreover, were observed with the gamma-law by Kolmogorov's test (the critical probability was not below 0.99). Irradiation changed the role of subordination of distributions of mitochondrial areas. In this case the gamma-distribution approximated to the experimental data much less well. For instance, after 30 min the distribution was in fact normal, but after 4 and 5 h it was lognormal. In the other cases, although the gamma-distribution was the best of the chosen theoretical laws, nevertheless critical probability for their approximation was too low. Since in most cases the gamma-distribution approximates better in mitochondria isolated from hepatocytes of normal and irradiated animals, and has the form:

$$f_{(a,\,b)}(\chi) = \frac{b^a}{\Gamma(a)}\,\chi^a - 1l - ex$$

at $0 \leqslant \chi < \infty$, the parameters of these laws can be regarded as fundamental informative parameters of the change in state of the liver mitochondria in irradiated animals (Table 3).

Testing the homogeneity of random samples of mitochondria showed that in all the experiments involving irradiation, significant differences in the distribution of mitochondria by size were present compared with the control experiment, whereas in seven experiments performed on unrradiated animals, the differences were for practical purposes not significant.

Thus the statistical analysis of data on changes in size of isolated mouse liver mitochondria showed that irradiation leads to an increase in their size, which is reflected in the character of the distributions. These changes can be associated with mitochondrial function, and changes in the distributions with the manifestation of the powers of adaptation of the hepatocytes.

LITERATURE CITED

1. M. S. Abramovich, L. S. Vorob'ev, N. A. Zmachinskaya, et al., in: Computer Programs [in Russian], No. 44, Minsk (1983), Part 2, pp. 119-131.
2. G. G. Avtandilov, V. V. Sirotkin, and E. V. Kozyreva, Byull. Eksp. Biol. Med., No. 8, 96 (1981).
3. E. V. Kozyreva, V. M. Mityushin, and V. V. Sirotkin, in: Regulation of Energy Metabolism and the Physiological State of the Organism [in Russian], Moscow (1978), pp. 173-176.
4. N. K. Monakhov, Mol. Biol., No. 1, 114 (1967).
5. V. V. Sirotkin, in: Statistical Properties of Microstructures [in Russian], Moscow (1978), pp. 192-193.