LITERATURE CITED

1. L. A. Knyazeva, D. A. Kozlov, P. V. Sutyagin, et al., Byull. Eksp. Biol. Med., No. 7, 108 (1981).
2. A. S. Pylaev, V. B. Turovetskii, and L. A. Knyazeva, Byull. Éksp. Biol. Med., No. 7, 114 (1985).
3. H. Alho, M. Partanen, J. Koistinaho, et al., Histochemistry, 80, 457 (1984).
4. R. E. Coupland, The Natural History of the Chromaffin Cell, London (1965).
5. O. Eränkö and S. Soinila, J. Neurocytol., 10, 1 (1981).
6. M. Lempinen, Acta Physiol. Scand., 62, 231 (1964).
7. M. Partanen, I. Linnola, A. Hervonen, and S. I. Rapoport, Neurobiol. Aging, 5, 105 (1984).
8. S. Soinila, J. Auton. Nerv. Syst., 11, 207 (1984).
9. J. Taxi, Int. Rev. Cytol., 57, 283 (1979).
10. R. Yokota and G. Burnstock, Cell Tissue Res., 192, 187 (1978).

EFFECT OF IRRADIATION ON DIMENSIONS OF RAT CARDIOMYOCYTE MITOCHONDRIA

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Correlation between the redistribution of dimensions of mitochondria and the biochemical parameters of their function [2, 5, 6] has been demonstrated by morphometric analysis of isolated liver mitochondria [7-11]. The time course of the changes in their size enables empirical data to be approximated by a theoretical gamma law of distribution [3]. It has been concluded from investigations that the parameters of this law of distribution, characterizing changes in the size of isolated liver mitochondria from irradiated control animals, are basic informative parameters of the state of the mitochondria, and it has now become possible to judge the functions of these organelles objectively by a study of electron-microscopic images.

The aim of this investigation was to study the particular features of rat cardiomyocyte mitochondria on the basis of changes in their size in response to external factors.

EXPERIMENTAL METHOD

Analysis was made of 16 series of experiments in which the dimensions of cardiomyocyte mitochondria were determined in 42 noninbred male albino rats weighing from 150 to 250 g. The first series of experiments constituted the control, namely cardiomyocyte mitochondria from intact, nonirradiated animals. In 13 series single irradiation in various doses was used: three series of experiments with a dose of 6 Gy, three series with a dose of 9 Gy, and seven series with a dose of 20 Gy. In three series of experiments, with a single dose of irradiation of 6 Gy, random samples of cardiac mitochondria were studied 2 and 24 h and 5 days after irradiation. In three series of experiments with irradiation in a dose of 9 Gy, cardiac mitochondria were studied after the same time intervals (2 and 24 h and 5 days). In seven series of experiments with irradiation in a dose of 20 Gy, cardiomyocyte mitochondria were analyzed after the following time intervals: 2 h, 1, 5, and 10 days, and 1, 6, and 12 months. The rats were irradiated on the TKhN-250 x-ray therapy apparatus, by means of a specially devised method of focusing the beam on the heart region [4]. Specimens of hearts for study were prepared for examination in the electron microscope by known methods, with fixation in buffered

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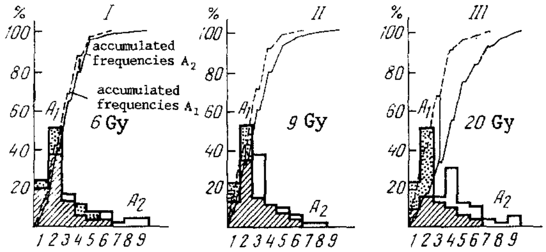

Fig. 1. Assessment of divergence of distributions of cardiomyocyte mitochondria by area depending on dose of irradiation. Abscissa, classes of mitochondria. Ordinate, frequency and accumulated frequencies. Oblique shading indicates common area of distribution of intact (A_1) and irradiated (A_2) mitochondria; unshaded part indicates area of distribution A_1 not common to both; arrows indicate distributions equal to area of distribution A_1 not common to both.

TABLE 1. Pearson's and Kolmogorov's Tests of Goodness of Fit after Comparison Empirical with Theoretical Data, Relative to Three Laws of Distribution of Areas of Cardiomyocyte Mitochondria

No. of series of experiments	Time of experiment	Pearson's criterion of goodness of fit (χ^2)			Kolmogorov's criterion of goodness of fit (λ)			Critical probability of gamma distribution
		normal distribution	log normal distribution[1]	gamma law of distribution	normal distribution	lognormal distribution	gamma law of distribution	
				Control				
1	2 h	13,39	—	2,69	0,72	0,88	0,43	0,99
2	1 day	14,16	—	1,87	0,84	0,83	0,37	1
3	5 days	11,05	—	0,95	0,87	0,69	0,28	1
				Irradiation in a dose of 6 Gy				
4	2 h	37,94	—	0,94	1,64	0,4	0,55	0,93
5	1 day	24,38	—	12,62	1,61	0,81	0,63	0,82
6	5 days	10,33	—	2,6	0,73	0,65	0,30	1
				Irradiation in a dose of 9 Gy				
7	2 h	19,41	—	7,06	1,45	0,5	0,65	0,78
8	1 day	14,07	—	4,05	1,37	0,17	0,43	0,99
9	5 days	18,50	—	0,45	1,05	0,68	0,23	1
				Irradiation in a dose of 20 Gy				
10	2 h	46,59	—	7,92	1,95	0,44	0,74	0,64
11	1 day	—	—	2,15	0,88	0,13	0,41	1
12	5 days	30,69	—	15,79	1,99	0,66	1,06	0,21
13	10 days	19,97	—	23,27	1,92	1,11	0,89	0,41
14	1 month	12,28	—	2,97	0,99	0,24	0,32	1
15	6 months	18,18	—	1,75	1,23	0,34	0,31	1
16	12 months	14,41	—	6,57	1,42	0,29	0.62	0,84

Legend. [1]Statistic and probability of Pearson's χ^2 criterion not calculated because the number of degrees of freedom is negative.

glutaraldehyde and osmium tetroxide solution. Sections through cardiomyocytes were examined in the JEM-7A electron microscope. The morphometric investigation was conducted by the method suggested previously [7-11], whereby the mitochondria were divided into classes. Just as in experiments with isolated mouse liver mitochondria [8], in the present investigation mitochondria of the control series were divided into six classes, whereas in the series of experiments with irradiation, an additional three classes were introduced on account of the increase in area of the mitochondria. The hypotheses relating to laws of distribution of the experimental data were tested with theoretical (normal, lognormal, and gamma laws of distribution) by means of the RD-1 program [1], using Pearson's and Kolmogorov's criteria of significance.

EXPERIMENTAL RESULTS

On the basis of the data characterizing histograms of distribution of the dimensions of the mitochondria, and also of a sample of the greatest differences in the distributions of the mitochondria depending on dose, corresponding to the maximal value of χ^2, divergence of the

TABLE 2. Parameters of Gamma Law of Distribution of Areas of Cardiomyocyte Mitochondria of Normal and Irradiated Rats

No. of series of experiment	Time of experiment	Parameters of gamma law of distribution	
		parameter of scale (α)	parameter of shape (β)
Control			
1	2h	0,14±0,01	2,73±0,26
2	1 day	0,14±0,01	2,70±0,26
3	5 days	0,14±0,01	2,75±0,26
Irradiation in a dose of 6 Gy			
4	2 h	0,08±0,01	1,93±0,23
5	1 day	0,12±0,01	2,71±0,26
6	5 days	0,17±0,01	3,12±0,28
Irradiation in a dose of 9 Gy			
7	2 h	0,11±0,01	2,74±0,26
8	1 day	0,15±0,01	2,75±0,26
9	5 days	0,12±0,01	2,34±0,24
Irradiation in a dose of 20 Gy			
10	2 h	0,08±0,01	1,80±0,21
11	1 day	0,29±0,02	4,57±0,34
12	5 days	0,12±0,01	1,92±0,22
13	10 days	0,09±0,01	3,21±0,28
14	1 month	0,15±0,01	3,53±0,30
15	6 months	0,16±0,01	3,16±0,28
16	12 months	0,16±0,01	2,85±0,27

distributions of the cardiomocyte mitochondria with respect to their areas and depending on the dose of local irradiation of the rats' hearts, was estimated (Fig. 1). Divergence of distributions of mitochondrial areas for control and irradiated animals reached its peak value with irradiation in a dose of 20 Gy. Curve A_1 characterizes the distribution of areas of cardiomyocyte mitochondria of intact hearts, whereas curve A_2 shows the distribution of cardiomyocyte mitochondria of irradiated animals. The total area of the distributions of the intact and irradiated mitochondria is shaded on the graphs. The continuous broken line represents the integral curve of accumulated frequencies of A_1, whereas the interrupted broken line represents accumulation of frequency A_2. The maximal distance between them, indicated by an arrow, is evidence that with an increase in the dose of irradiation there is a corresponding increase of divergence between the distributions of mitochondria in the control and irradiated samples, numerically equal to the area of distribution A_1 which is not common to both. This is shown by the decrease in the common area of the distributions (the obliquely shaded part of the histograms). Analysis of the histograms shows how the frequency of accumulation of mitochondria in each particular class changes. During irradiation of the rats' hearts in a dose of 6 Gy (Fig. 1, I) maximal accumulation of the number of mitochondria relates to class 2. In the control sample some decrease was observed in the number of mitochondria in classes 1 and 3, on average by 6%. During irradiation in a dose of 9 Gy (Fig. 1, II) the maximum of the histogram of distribution was shifted from class 2 to class 3, where their frequency was significantly higher than in the control (it rose from 23 to 34%), the number of large mitochondria remaining at a high level. An increase in the dose of irradiation to 20 Gy shifted the maximum of the distribution to class 4 (Fig. 1, III), where the number of mitochondria increased from 11 to 28%, and further accumulation took place in the large classes [7-9]. The change in functional activity during irradiation can be explained by redistribution of the areas of the mitochondria, a change in the relative numbers of mitochondria with different sizes, and an increase in the relative number of large mitochondria with depressed respiratory function [2, 3]. Qualitative ultrastructural analysis showed that an increase in the bulk density of the rat cardiomyocyte mitochondria in the early stages after irradiation reflects the destructive stage in the response of the mitochondria to the damaging factor and is connected with an increase in their size due to excessive swelling. After irradiation, maximal deviations of the quantitative and qualitative characteristics of the structural elements of the mitochondria and myocardium studied were found to alternate with minimal deviations in the trend of development of the postulated effect.

Statistical analysis of the results of measurement of the cardiomyocyte mitochondria and testing agreement between the empirical and theoretical (normal, lognormal, and gamma law) distributions by Pearson's and Kolmogorov's test showed that in all three series of control experiments the empirical distributions were approximated by the gamma law of distribution. The values of χ^2 and λ obtained by comparison of the empirical data with the theoretical relative to normal, lognormal and gamma laws of distribution, shown in Table 1, confirm this conclusion. No significant differences were found with the gamma law by Kolmogorov's test: The critical probability was not below 0.9. In the series of experiments with irradiation the critical probability decreased with an increase in the dose of irradiation. However, considering that in all the control series of experiments to study distributions of areas of mitochondria, the distribution was approximated by the gamma law, whereas in series of experiments with irradiation, the gamma distribution in most cases approximated better to the experimental data, the parameters of this law must be considered to be the principal informative parameters of the change in state of the cardiomyocyte mitochondria in irradiated and control animals (Table 2), and the histograms characterizing distributions of mitochondria can be described by the following mathematical relationship:

$$f(x) = \frac{1}{\alpha!\beta^{\alpha+1}} \chi^{\alpha} e^{-\chi/\beta} \text{ when } 0 \leqslant \chi \leqslant \infty,$$

i.e., $f(x) = f\chi, \alpha, \beta$,

where α is the parameter of shape and β the parameter of scale, characterizing distributions of the quantitative ratio between different types of mitochondria.

Testing the homogeneity of random samples of areas of rat heart mitochondria in the control series and in series with local irradiation of the heart in different doses showed that there were significant differences from the control series in the distributions of mitochondria by size in all series of experiments, but in series of experiments on animals which were not irradiated the differences were not significant.

The established changes in the relative proportions of mitochondria with an increased number of large organelles under the influence of irradiation may thus be evidence of their functional insufficiency, and may probably be the cause of the energy deficiency of the cells. An increase in the number of small mitochondria can be regarded as a manifestation of increased activity of the compensatory and adaptive mechanisms of the energy supply to subcellular structures. Considering the rule that sizes of mitochondria obey a gamma law, revealed by this investigation, both for isolated hepatocyte mitochondria and for cardiomyocyte mitochondria, it is our opinion that the functional state of the mitochondria can be judged on the basis of changes in their distribution by size. Accordingly, there are good grounds for undertaking a morphometric assessment of changes in the dimensions of mitochondria during electronmicroscopic study of cells in order to evaluate the morphological and functional properties of these cellular organelles, and of the cell as a whole.

LITERATURE CITED

1. M. S. Abramov, L. S. Vorob'ev, N. A. Zmachinskaya, et al., Computer Software [in Russian], No. 44, Part 2, Minsk (1983), pp. 119-131.
2. G. G. Avtandilov, V. V. Sirotkin, and E. V. Kozyreva, Byull. Éksp. Biol. Med., No. 8, 96 (1981).
3. G. G. Avtandilov, V. V. Sirotkin, M. Yu. Dovzhenko, and A. P. Lobzin, Byull. Éksp. Biol. Med., No. 4, 409 (1986).
4. M. Ya. Bogomazov and F. F. Krotkov, Modeling, Methods of Study, and Experimental Therapy of Pathological Processes [in Russian], Moscow (1973), pp. 27-28.
5. E. V. Kozyreva, V. M. Mityushin, and V. V. Sirotkin, Regulation of Energy Metabolism and the Physiological State of the Organism [in Russian], Moscow (1978), pp. 173-176.
6. V. M. Mityushin, V. V. Sirotkin, and E. V. Kozyreva, Proceedings of the 9th All-Union Conference on Electron Microscopy [in Russian], Moscow (1973), pp. 389-391.
7. V. V. Sirotkin, Statistical Properties of Microstructures [in Russian], Moscow (1978), pp. 192-193.
8. V. V. Sirotkin, Med. Ref. Zh., 22, No. 12 (1981).
9. V. V. Sirotkin, N. P. Lebkova, and G. G. Avtandilov, Author's Certificate No. 1086988/USSR (1983).
10. V. V. Sirotkin, G. G. Avtandilov, V. A. Kobylyakov, and T. P. Gladkikh, Author's Certificate No. 1151143/USSR (1984).
11. V. V. Sirotkin and A. N. Martynov, Author's Certificate No. 1314875/USSR (1987).