tabolism and weakening of its regulatory role in the central nervous system during the aging process.

REFERENCES

1. I. I. Miroshnichenko, V. S. Kudrin, and K. S. Raevskii, *Farmakol. Toksikol.*, No. 2, 26-29 (1988).
2. V. V. Frol'kis *et al.*, *Aging of the Brain* [in Russian], Leningrad (1991).
3. I. Date, D. L. Felten, and S. Felten, *Brain Res.*, **519**, 266-276 (1990).
4. D. E. Dluzen, J. L. McDermott, and V. D. Ramirez, *Exp. Neurol.*, **106**, 259-264 (1989).
5. F. Godefroy, M. H. Bassant, Y. Lamour, and J. Weil-Fugazza, *J. Neural Transmis.*, **83**, 13-24 (1991).
6. I. M. Henry, G. S. Joseph, K. Kochman, *et al.*, *Brain Res.*, **418**, 334-342 (1987).
7. L. Oreland, in: *Normal Aging, Alzheimer Disease, and Senile Dementia*, Brussels, (1985), pp. 129-134.
8. H. H. Osterburg, H. G. Donahue, J. A. Severson, *et al.*, *Brain Res.*, **224**, 337-352 (1981).
9. F. Ponzio, G. Gladerini, G. Lomuscio, *et al.*, *J. Neurobiol. Aging*, **3**, 23-29 (1982).
10. J. L. Venero, A. Machado, and J. Cano, *Brain Res.*, **557**, 109-114 (1991).
11. J. J. Woodward, S. W. Leslie, J. A. Severson, and R. E. Wilcox, *Neurosci. Lett.*, **97**, 191-197 (1989).
12. T. Zetterstrom, T. Sharp, A. K. Collin, and U. Ungerstedt, *Ibid.*, **148**, 327-334 (1988).
13. T. Zetterstrom, T. Sharp, and U. Ungerstedt, *Europ. J. Pharmacol.*, **132**, 1-9 (1986).

Three-Factor Correlation Analysis of Morphofunctional Changes of Mitochondria during Irradiation

V. V.Sirotkin, A. Yu. Grishin, V. A. Smirnov

UDC 615.849.1.015.44.076.9

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol.115, № 3, pp. 303–305, March, 1993
Original article submitted October 21, 1992

Key Words: *morphofunctional changes of mitochondria; correlation analysis; correlation coefficient; closeness and connection of correlation; regression*

There have been many studies of structural and functional changes of animal and plant mitochondria (Mt) under conditions of dosed exposures at different time intervals. However, strict mathematical analysis has rarely been used, and the results are often subjective, only to be taken as hypotheses requiring further investigation and corroboration. One proof can be the establishment of a correlation between test variables such as doses of exposures, time intervals, and morphological and functional characteristics of the biological object. Multifactorial correlation analysis and calculations of the correlation coefficients should be performed, to reveal these connections. For instance, due to the results of previous two-factor correlation analysis between known morphofunctional mitochondria types and redistribution of Mt sizes, we obtained supplementary proofs of our earlier conclusions [3-5]: knowing the areas of Mt, it is possible to determine their functional properties with a high degree of reliability, and, conversely, knowing the functional properties, it is possible to judge the areas using regression lines to estimate the whole population of Mt [8].

The present study aims at proving the existence of a correlation between such variables as time period, rate of respiration and redistribution of Mt ar-

Institute of Physics and Technical Problems, Moscow
(Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007–4888/93/0003–0333$12.50

eas, examined after a one-trial irradiation of experimental animals.

MATERIALS AND METHODS

The experiments were carried out on 70 CBA3C57Bl hybrid male mice of the same age and weight (22 g). Each experimental series consisted of hepatocyte Mt of 5 animals. The isolation of Mt was performed simultaneously for the control, normal and one-trial irradiation-treated (dose 7,5 Gy) groups. The time intervals were 5 min, 30 min, and 1, 2, 3, 4, and 5 hours for each experimental series. Mt isolation using the method of differential centrifugation and polarographic assay of Mt respiration was performed according to a method described previously [1]. For morphometric study of Mt area redistribution a suspension of organelles after the termination of the Mt respiration cycle was used (the fourth Chance state). The most marked variations in Mt respiration are observed in the third Chance state (i.e., for phosphorylation of added ADP), and therefore the graph (Fig.1) and Table 1 present data of the Mt respiration rate of the irradiated animals to this stage. Morphometry was performed according to our method,as described repeatedly [4,5,7]. In this method the values of Mt areas are connected to form continuous variational series and, using Stendgers' formula that takes into account minimal and maximal values, are separated into groups. We examined the dependence of the respiration rate (v) on two factors: time period (t) and Mt area (S). It is to be noted that the respiration rate depends linearly on both the time period and the Mt area. Hence it follows that the respiration rate, being a function of two variables, time and Mt areas, is also linear:

$$v = at + bS.$$

In the case of irradiated animals the empirical data and group means of Mt areas obtained after correlation analysis were used. Group means are arithmetic means of variables (5, 15, 25, 35,...85 mm^2) measured by electron microscopic examination (з10,000).

RESULTS

All empirical data are listed in Table.
The equation of multifactorial correlation is as follows:

$$v = at + bS + c.$$

In our case:

$$v = \bar{v} + a(t - \bar{t}) + b(S - \bar{S}).$$

t_i, h	S_i, mm^2	v_i	n_i
1/12	19.467	1.00	150
1/2	35.533	0.36	150
1	22.533	0.48	150
2	33.467	0.50	150
3	17.267	0.20	150
4	24.867	0.22	150
5	26.333	0.34	150
Σn_i	–	–	1050

Coefficients a and b will be found using the correlation coefficients between pairs of variables $\bar{t}$ and $\bar{S}$, t and v, and S and v:

$$r_{tS} = \frac{\overline{tS} - \bar{t} \cdot \bar{S}}{\sigma_t \cdot \sigma_S}; \quad r_{tv} = \frac{\overline{tv} - \bar{t} \cdot \bar{v}}{\sigma_t \cdot \sigma_v}; \quad r_{Sv} = \frac{\overline{Sv} - \bar{S} \cdot \bar{v}}{\sigma_S \cdot \sigma_v}.$$

So [2] :

$$a = \frac{r_{tv} - r_{tS} \cdot r_{Sv}}{1 - r_{tS}^2} \cdot \frac{\sigma_v}{\sigma_t}; \quad b = \frac{r_{Sv} - r_{tv} \cdot r_{tS}}{1 - r_{tS}^2} \cdot \frac{\sigma_v}{\sigma_S}.$$

In these formulas

$$\sigma_t = \sqrt{\frac{\sum_{i=1}^{9} (t_i - \bar{t})^2 \cdot n_i}{n}}; \quad \sigma_S = \sqrt{\frac{\sum_{i=1}^{9} (S_i - \bar{S})^2 \cdot n_i}{n}};$$

$$\sigma_v = \sqrt{\frac{\sum_{i=1}^{9} (v_i - \bar{v})^2 \cdot n_i}{n}}.$$

For all these calculations we need the arithmetic means of variables t, S, and v and their paired products: $\bar{t}$, $\bar{S}$, $\bar{v}$, $\overline{tS}$, $\overline{tv}$, $\overline{Sv}$. Let us find them:

$$\bar{t} = \frac{\sum_{i=1}^{9} t_i \cdot n_i}{n} = \frac{150(\frac{1}{12} + 0.5 + 1 + 2 + 3 + 4 + 5)}{1050} = 2.226;$$

$$\bar{S} = \frac{\sum_{i=1}^{9} S_i \cdot n_i}{n} = \frac{150(10.457 + 35.533 + 22.533 + \rightarrow}{1050}$$

$$\rightarrow \frac{+33.467 + 17.267 + 24.867 + 26.33)}{1050} = 25.638;$$

$$\bar{v} = \frac{\sum_{i=1}^{9} v_i \cdot n_i}{n} = \frac{150(1.00 + 0.36 + 0.48 + 0.50 + \rightarrow}{1050}$$

$$\rightarrow \frac{+0.20+0.22+0.34)}{} = 0.443.$$

$$\overline{tS} = \frac{\sum_{i=1}^{9} t_i \cdot S_i \cdot n_i}{n} = 55.97;$$

$$\overline{tv} = \frac{\sum_{i=1}^{9} t_i \cdot v_i \cdot n_i}{n} = 0.703;$$

$$\overline{Sv} = \frac{\sum_{i=1}^{9} S_i \cdot v_i \cdot n_i}{n} = 11.098.$$

Now let us find the standard deviations:

$$\sigma_t = 1.714; \quad \sigma_S = 6.303; \quad \sigma_v = 0.252.$$

Substituting the values obtained, we can calculate the correlation coefficients and then the coefficients of the equation:

$$r_{tS} = \frac{55.97 - 2.226 \cdot 25.638}{1.714 \cdot 6.303} = -0.102;$$

$$r_{tv} = \frac{0.703 - 2.226 \cdot 0.443}{1.714 \cdot 0.252} = -0.655;$$

$$r_{Sv} = \frac{11.098 - 25.638 \cdot 0.443}{6.303 \cdot 0.252} = -0.163;$$

$$a = \frac{-0.655 - (-0.102) \cdot (-0.163)}{1 - (-0.102)^2} \cdot \frac{0.252}{1.714} = -0.1;$$

$$b = \frac{-0.163 - (-0.655) \cdot (-0.102)}{1 - (-0.102)^2} \cdot \frac{0.252}{6.303} = -0.01.$$

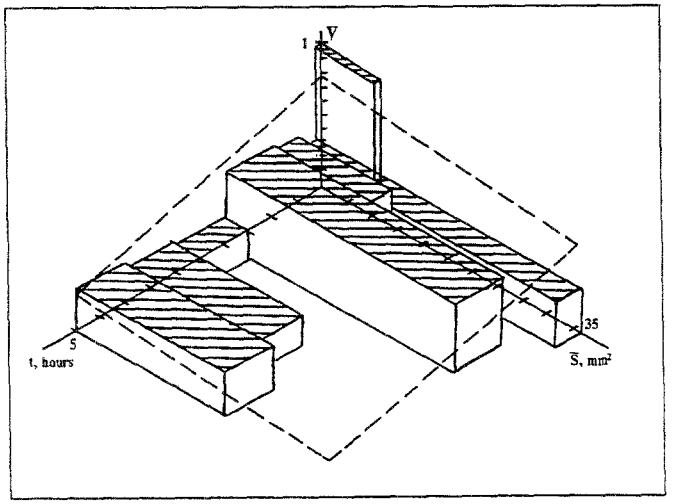

Fig. 1. Plot of the empirical data and of the regression plane of the respiration, time, and of the group mean of Mt area.

In sum:

$$v = 0.443 - 0.1(t - 2.226) - 0.01(S - 25.638);$$

$$v = 0.443 - 0.1 \cdot t + 0.223 - 0.01 \cdot S + 0.256;$$

$$v = -0.1 \cdot t - 0.01 \cdot S + 0.922.$$

The boundaries of the plane are shown on the histogram by a dotted line (Fig. 1). To establish the closeness of the correlation connection, let us find the combined correlation coefficient R [2]:

$$R = \sqrt{\frac{r_{tv}^2 + r_{sv}^2 - 2 \cdot r_{tv} \cdot r_{Sv} \cdot r_{tS}}{1 - r_{tS}^2}};$$

$$R = \sqrt{\frac{(-0.655)^2 + (-0.163)^2 - 2 \cdot (-0.655)}{1 - (-0.102)^2}} \times$$

$$\times \sqrt{\frac{(-0.163) \cdot (-0.102)}{1 - (-0.102)^2}} = 0.655 \approx 0.7$$

As R=0,7 (close to unity), it may be deduced that the Mt respiration rate in irradiated animals is closely correlated with S and t.

Besides, as R is not equal to 1, the correlation of v with t and S cannot be described as a precise linear function, but R is not 0, and so we can use the linear correlation: $v=f(t,S)$, that is our equation v=-0,1t-0,01S+0,922. The empirical values of the respiration rate, dependent on t and S, are displaced somewhat from this theoretical plane.

Now let us estimate the separate influence of t and S on the respiration rate. The specific correlative coefficient between v and t $r_{tv(S)}$ evaluates the strength of the linear correlation between v and t, when S remains constant. The strength of the linear correlation between v and S, when S remains constant, is estimated using the specific correlation coefficient between S and v:

$$r_{Sv(t)} = \frac{r_{Sv} - r_{tS} \cdot r_{tv}}{\sqrt{(1 - r_{tS}^2) \cdot (1 - r_{tv}^2)}};$$

$$r_{tv(S)} = \frac{(-0.655) - (-0.102) \cdot (-0.163)}{\sqrt{(1 - (-0.102)^2) \cdot (1 - (-0.163)^2)}} =$$

$$= -0.682 \approx -0.7;$$

$$r_{Sv(t)} = \frac{(-0.163) - (-0.102) \cdot (-0.655)}{\sqrt{(1 - (-0.102)^2) \cdot (1 - (-0.655)^2)}} =$$

$$= -0.306 \approx -0.3.$$

The obtained values of the specific correlation coefficient demonstrated the strong dependence of the

respiration rate v on the time interval in the case of constant Mt area S=const, whereas the dependence on Mt area under constant time t = const is expressed weakly. The combined correlation gives a more accurate description of the dependence of Mt activity on both factors at once and leads to a number of conclusions.

Thus, 1) the Mt respiration rate can be described by a linear correlation with the time period and Mt area; 2) negative coefficients in the equations indicate that the respiration rate drops both with an increase of the time and with augmentation of the Mt area; 3) the equations obtained permit an estimatation of the state of the Mt population according to Mt sizes and the time period and show that the larger the Mt is, the shorter life it has; 4) it may be assumed that the rate of Mt mortality will depend on the dose of radiation, and this leads to changes of the equation coefficients.

Probably, further investigations will prove or refine our conclusions. This calls for appropriate studies using the proposed scheme of correlation analysis. In our view, such an approach makes the evaluation of morphofunctional relationships in medical and biological assays more objective.

We are indebted to Dr. N.A.Yurina for her help in the discussion of some aspects of this investigation.

REFERENCES

1. G. G. Avtandilov, V. V. Sirotkin, and E. V. Kozyreva, *Byull. Eksp. Biol.*, **92**, № 8, 96-97 (1981).
2. A. I. Karasev, *Probability Theory and Mathematical Statistics* [in Russian], Moscow (1979).
3. V. M. Mityushin, V. V. Sirotkin, and E. V. Kozyreva, *Ninth Conference on Electron Microscopy*, Moscow (1973), pp. 389-390.
4. V. V. Sirotkin, in: *Statistical Properties of Microstructures* [in Russian], Moscow (1978), pp. 192-193.
5. V. V. Sirotkin, *Med. Ref. Zh.* **XXII**, № 12, (1985).
6. V. V. Sirotkin, *Method of Estimating the Morphofunctional of Mitochondria State, Pat. № 14887200 SSSR* (1989).
7. V. V. Sirotkin and N. D. Volodin, *Byull. Eksp. Biol.*, **110**, № 7, 100-103 (1990).
8. V. V. Sirotkin, A. Yu. Grishin, V. A. Smirnov, *et al.*, *Byull. Eksp. Biol.*, **115**, № 2, 215-218 (1993).

Electron-Microscopical Study of Cardiomyocyte Chromatin in Epinephrine-Treated Dogs

R. I. Klyasheva

UDC 576.3.315.42

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 305–307, March, 1993
Original article submitted April 13, 1992

Key Words: *cardiomyocytes; chromatin rearrangement; epinephrine; nucleoplasmic chromatin; perimembrane chromatin*

It is well known that one of the key pathogenic mechanisms switched on by extreme conditions is endogenous epinephrine, the boosted synthesis and release of which into the blood leads to a hypertoxic effect on the cardiomyocytes (CM), resulting in a significant drop of the level of DNA methylcytosine (5-MC). Biological methods [4,5] previously used by us are associated with the study of DNA methylation by cytosine.

In the present work we studied the chromatin ultrastructure and, in particular, the area of dense

Department of Pharmacology, I. N. Ul'yanov State University, Chuvash. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007–4888/93/0003–0336$12.50