LITERATURE CITED

1. A. D. Bershadskii (Bershadsky), V. I. Gel'fand (Gelfand), T. M. Svitkina, et al., Exp. Cell Res., 127, 423 (1980).
2. A. Di Pasquale, Exp. Cell Res., 95, 425 (1975).
3. A. Harris, Ciba Foundation Symp., 14, 3 (1973).
4. J. Heath and G. A. Dunn, J. Cell Sci., 29, 197 (1978).
5. C. S. Izzard and L. R. Lochner, J. Cell Sci., 21, 129 (1976).
6. E. Lazarides, J. Histochem. Cytochem., 23, 507 (1975).
7. J. Wehland, M. Osborn, and K. Weber, J. Cell Sci., 37, 257 (1979).
8. I. Yahara and F. Kakimoto-Sameshima, Proc. Natl. Acad. Sci. USA, 74, 4511 (1977).

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF MOUSE LIVER MITOCHONDRIA (MORPHOLOGICAL AND BIOCHEMICAL STUDY)

G. G. Avtandilov, V. V. Sirotkin, and E. V. Kozyreva

UDC 612.35.014.21

KEY WORDS: quantitative analysis; mitochondria; electron microscopic investigation; irradiation; respiration and oxidative phosphorylation; polarography.

Mainly qualitative criteria of assessment are used in electron-microscopic investigations of cell organelles. However, some aspects of submicroscopic cytology can be investigated by quantitative methods of morphometry, which can be used to evaluate electron-micrographs [4, 5].

The object of this investigation was to study correlation between the dimensions of intact mitochondria and biochemical parameters of their activity.

EXPERIMENTAL METHOD

Hybrid male CBA × C57BL mice of the same age and weight (22 g) were used. Each point was represented by five mice. Mitochondria were isolated from the liver on two centrifuges (parallel for the control, normal state, and after γ-ray irradiation of the animals in a dose of 750 R at a dose rate of 641 R/min). Time in the experiment was counted from irradiation (5, 30, and 60 min after irradiation, thereafter every 60 min until 8 h). The experiment included two repetitions. 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. Respiration of the mitochondria was studied by a polarographic method, using the PO-4 polarograph (Denmark) with rotating platinum electrode in a 1-ml cell at 26°C. The composition of the incubation medium was: 0.2 M sucrose, 0.03 M Tris buffer, 0.01 M $MgCl_2$, 0.015 M KCl, 0.02 M KH_2PO_4, 0.2 mM EDTA, pH 7.4. The oxidation substrate was succinic and glutamic acids (5 mM). ADP (0.15 mM) was added. For counting the mitochondria a suspension of organelles was used after completion of their respiration cycle in a polarographic cell (i.e., in fact in stage 4 after Chance). Mitochondrial respiration showed the greatest fluctuations in Chance's state 3 (i.e., during phosphorylation of the added ADP), and for that reason data on the rate V3 of mitochondria isolated from the liver of irradiated mice are plotted on the graph in Fig. 1. The area of the mitochondria, calculated by the equation $S = \pi ab$, where S is the area of the mitochondria, their smallest radius, and b their greatest radius, was used as the morphometric test for assessing the state of the mitochondria. Altogether 1500 mitochondria were analyzed; a suspension of them was applied to a grid and negatively stained with 10% phosphotungstic acid solution.

The results of calculations for the test mitochondria were reduced to continuous variance series and broken down into classes by means of Stendzhes' equation $i = \frac{x_{max} - x_{min}}{1 + 3.32 \ln h}$, in which i is the class interval, x_{max} the maximal variant,

Department of Pathological Anatomy and Central Research Laboratory, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 96-97, August, 1981. Original article submitted January 30, 1981.

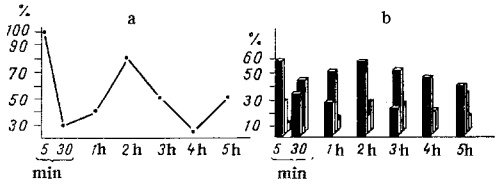

Fig. 1. Changes in rate of respiration of liver mitochondria (a) from irradiated mice (in Chance's state 3) and dynamics of changes in relative proportions of mitochondria of different sizes (extensive values) at different times after irradiation (b). Abscissa, time after irradiation.

x_{min} the minimal variant of the set, ln h is the logarithm to base 10 of the number of variants in a given set. In the present experiment nine classes were distinguished, in which mitochondria were distributed according to the increase in areas. Later, for convenience of analysis, the classes were combined into three groups. Since different sets had to be dealt with and compared with each other, the number of frequencies expressed as a percentage of the number of variants of a given set was used in the analysis. The mean area was calculated as the simple arithmetic mean by the equation

$\bar{x} = \frac{\Sigma xi}{n}$ [1, 2].

The following graphs were plotted from an analysis of the results of measurements of the mitochondria and the class intervals: changes in the relative percentages of mitochondria of different sizes in the population, isolated at different moments of time after irradiation; changes in the mean area of the mitochondria depending on the time after irradiation; changes in mitochondria by classes depending on the time after exposure; changes in the mitochondria by groups depending on the time after irradiation; the dynamics of changes in the large mitochondria; changes in mitochondria by classes in connection with changes in their respiration.

EXPERIMENTAL RESULTS

A study of histograms of the distribution of mitochondria at definite times after irradiation showed a distinct regular pattern. In the control accumulation of mitochondria was observed mainly in the first three classes, which accounted for 75-95% of all mitochondria. A tendency was observed in the experiment for the size of the mitochondria to increase, and their number in the first three classes varied over a wide range (from 36 to 83%). A sharp decrease was observed 5 min after irradiation in the area of the mitochondria and they accumulated in classes 1-3 (75%), whereas 30 min after irradiation the opposite response was found: 20% of the total number were left in classes 1 and 2. However, 1 h after the beginning of exposure concentration of the mitochondria in classes 1-3 was observed (55-70%). Comparison of these graphs shows that in the control mitochondria accumulated mainly in the central classes, with a gradual decrease in their number as the distance from the midpoint of the variance series increased. This rule, considered to be the characteristic feature of variance of biological characteristics (the law of distribution) was disturbed by irradiation. Additional maxima appeared on the graph, in classes 4 and 6 compared with the modal class 2. It can be concluded from these data that the mean area of all mitochondria in the control lies within one interval, whereas in the experiment a sharp decrease in the mean area of the mitochondria was observed 5 min after exposure compared with the control, and after 30 min the area was increased two-threefold, and it continued thereafter to keep the same parameters; these changes, moreover, were cyclic in character. The mean area of the mitochondria increased on account of an increase in the number of large mitochondria, which evidently swell and subsequently distintegrate. However, total degradation of all mitochondria was not observed.

It will be clear from Fig. 1b that with an increase in the number of mitochondria in group A (the first series of rectangles) an increase in the rate of phosphorylation was observed immediately. During an increase in the relative proportion of group C mitochondria (the third series of rectangles) the opposite relationship held well. These results show that the level or intensity of respiration of mitochondria in suspension is determined by the quantitative ratio between the numbers of mitochondria in different functional states; the leading role in respiration, moreover, belongs to group A mitochondria.

Respiration of the liver mitochondria followed a cyclic course with a phase of 3-4 h; the highest peak of activity and of the rate of respiration in state 3 was observed 5 min and 2-3 h after irradiation, and phases of decline in the rate of respiration were observed 30 min and 4 h after irradiation [3].

A similar redistribution of the classes of mitochondria may perhaps also take place in whole cells of tissues, and in that way the level of their energy metabolism is determined.

It can thus be concluded from these experiments that the method of negative staining of mitochondria followed by quantitative analysis of their electron-microscopic photographs is a sufficiently accurate and objective method of estimating

their size, which correlates also with their functional activity. Further research in this direction must evidently yield fresh data on the structural and functional states of mitochondria.

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. G. G. Avtandilov, Introduction to Quantitative Pathological Morphology [in Russian], Moscow (1980).
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. V. M. Mityushin, V. V. Sirotkin, and E. V. Kozyreva, in: Proceedings of the 9th All-Union Conference on Electron Microscopy [in Russian], Moscow (1973), pp. 389-391.
5. V. V. Sirotkin, in: Statistical Properties of Microstructures [in Russian], Moscow (1978), pp. 192-193.

STRUCTURE AND PROPERTIES OF TENDON COLLAGEN COMPLEX DURING DISORGANIZATION OF THE GROUND SUBSTANCE OF CONNECTIVE TISSUE

S. S. Nikolaeva, Yu. A. Khoroshkov, and V. A. Dubinskaya

UDC 616-018.2-07:616.75-008.939.629-074

KEY WORDS: carbohydrate-protein complexes; connective tissue.

An important place in human pathology is occupied by systemic diseases of connective tissue [4, 9, 11], accompanied by disorganization of the carbohydrate–protein complexes of the ground substance of connective tissue as a result of activation of mucolytic and proteolytic enzymes [5]. Under these circumstances the collagen fibers undergo essential changes [8, 11]. Analysis of the structure and properties of the collagen skeleton during enzymic disorganization of ground substance is of great interest for the closer study of the mechanisms of development of pathological changes in connective tissues affected by systemic diseases. On the basis of the facts indicated above it was decided to study the structure and properties of the collagen complex of tendons during enzymic disorganization of the ground substance.

EXPERIMENTAL METHOD

Achilles' tendons from persons aged 25-50 years, dying from trauma, were used as the test object. The material was obtained at autopsy within 24 h after death. Treatment with the enzyme amyloryzine was carried out for 6-12-24 h at 37°C, with an enzyme:substrate ratio of 1:25. A solution of the enzyme was made up in phosphate buffer, pH 5.6. Amyloryzine has no collagenase activity and is characterized by amylolytic and by a low level of proteolytic activity. The specimens of tendon also were treated with water for 24 h. The original specimens of tendon, and material treated with the enzyme or water, were investigated by scanning and transmission electron microscopy.

The physicochemical investigations of the enzyme-treated tendon were undertaken 24 h after the beginning of incubation. The glycosaminoglycan content was determined from the quantity of amino sugars [1]. Stretch diagrams of the specimens were studied in a medium of physiological saline on an elastodynamometer [10]. The temperature of hydrothermic contraction of the tendons also was determined on the same instrument. The modulus of elasticity was calculated from the tangent of the angle of slope of the rectilinear region of the curves. The contraction temperature of the tendons when heated in a dry state (20-220°C) was recorded on the apparatus described by Kaimin' [6]. The total moisture content and the quantity of hydration-bound water were determined by Fischer's method [7].

EXPERIMENTAL RESULTS

Fibrous connective tissue of normal tendon is a complex of collagen fibers, highly organized and combined into a single biological structure. The largest collagen fibers have a mutually parallel orientation, coinciding with the direction of the main mechanical stresses to which the tendon is subjected (Fig. 1). At the same time, the tendon also has a developed

Research Laboratory of Biological Structures, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 98-100, August, 1981. Original article submitted November 19, 1980.