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CHANGES IN FREEZE - FRACTURED MITOCHONDRIAL MEMBRANES DURING SPERMATOGENESIS

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ABSTRACT

The mitochondria of germ cells in spermatogenesis, and the transformation of mitochondria from an orthodox form in spermatogonia to a condensed form in spermatids has been described and widely characterized by TEM. The morphological correlation of freeze-fractured mitochondrial membranes with the energetic states of mitochondria has also been described. The frequency by which the fracture plane following the inner or outer boundary membrane deviates by jumping from one to the other is higher in phosphorylating mitochondria than in non - - phosphorylating mitochondria. The present study on replicas of freeze-fractured spermatogenic cells of the rat showed that the frequency of deflections of the fracture plane was lowest in spermatogonial mitochondria and increased in spermatocytes and spermatids. Our study seems to suggest that this method can be applied to tissue samples also after glutaraldehyde fixation.

KEY WORDS: freeze-fractured mitochondrial membranes, spermatogenesis.

INTRODUCTION

Studies of various authors using TEM (Andre, 1962; Cieciura and Klimek, 1988; Fawcett, 1970) reveal that during spermatogenesis and spermiogenesis, the changes of configuration of mitochanodrial membranes showed the transition from an orthodox state in spermatogonia through transitional steady states to condensed forms in spermatids. Combined morphological and biochemical studies have been performed on isolated germ cells and their mitochondria (Martino et al., 1976;1979) and show modification of the configuration of mitochondrial membranes from an orthodox (spermatogonia, spermatocytes to the stadium of late pachytene) to a condensed form (late pachytene, diplotene, secondary spermatocytes and spermatids from Golgi to the acrosomic phases) (Cieciura and Klimek, 1979). Several investigations have attempted to describe the configuration of mitochondrial membranes in various metabolic steady states in TEM as a spreading or condensing of the matrix; changes in volume of the outer and inner compartments; and changes of the thickness of the mitochondrial cristae (Chance and Williams, 1956; Cieciura et al., 1979; Hackenbrock, 1966;1968; Hackenbrock et al., 1971).

Knoll and Brdiczka (1983), described a morphological changes dependent on the metabolic state of the organelle in freeze-fractured isolated liver mitochondria. In the case of plane - fractured mitochondrial membranes they observed a change in the course of the fracture plane in two boundary membranes: the fracture plane changed between the interior of both boundary membranes. They observed that the fracture plane changes correlate with the metabolic state of mitochondrium. There are more deflections of the fracture plane in phosphorylating mitochondria than in non - phosphorylating ones (Figs. 1 a, b). These chan-

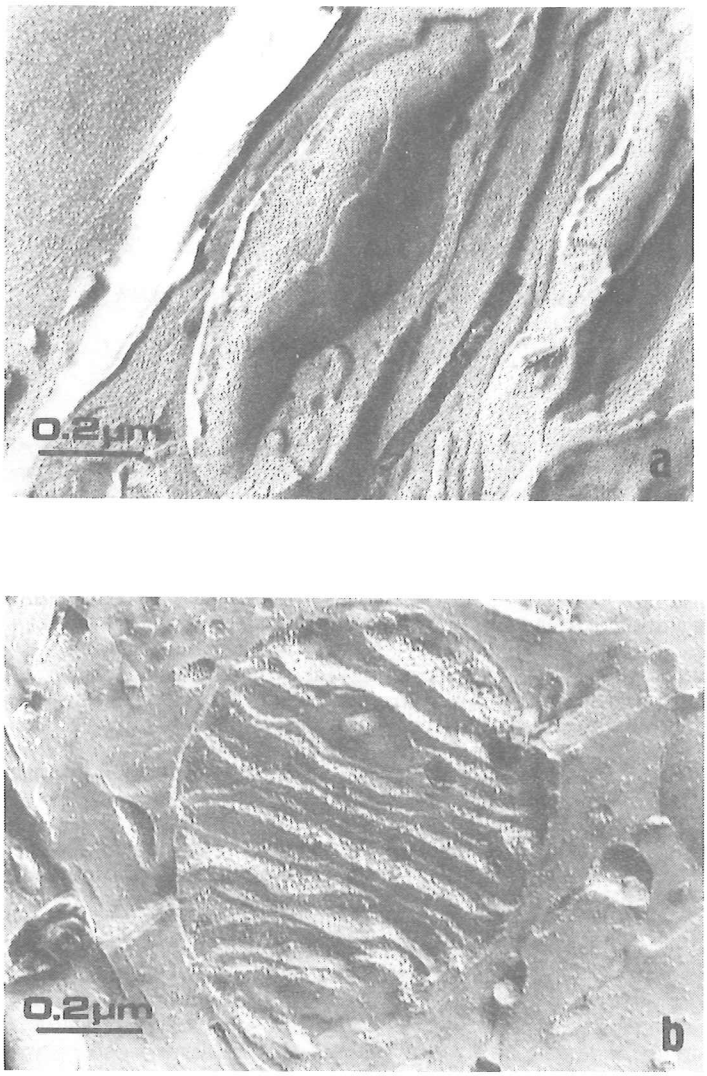

Figure 1. Etching of freeze-fractured mitochondria in orthodox state (a) and condensed state (b). In condensed mitochondria the deflections between boundary membranes are shown. In orthodox state number of deflections is decreased.

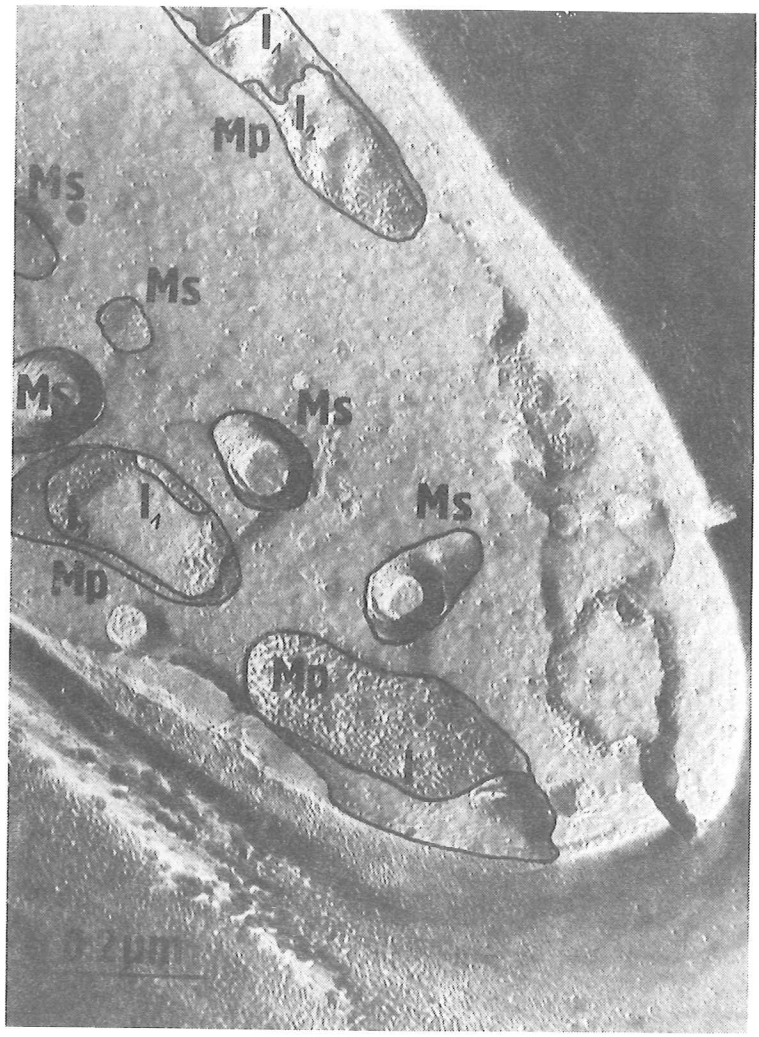

Figure 2. Freeze-fracture of mitochondria with a scheme of evaluation procedure. The length of the edge of the fracture plane deflection (as a sum of particular edges l_1, l_2, ... l_n) as related to the mitochondrion area was measured. The obtained value (L) was length per area. In order to perform an analysis which takes into consideration the different amount of smooth membrane fractures of mitochondria in the respective types of germ cells, the total area of smooth membrane fractures (Ms) and patchy membrane fractures (Mp) were measured. The single values L were weighted by the factor Mp(Mp + Ms) which is percentage of mitochondria with patchy faces in the whole mitochondrial population for the particular preparation.

ges of fracture plane deflections depend on contacts between the boundary membranes and their dynamic changes which are linked to the mitochondrial metabolism. Our aim in this present investigation is to attempt to determine the metabolic states in mitochondria of germ cells during spermatogenesis as assessed in freeze-fractured mitochondria following the principle Knoll and Brdiczka created.

MATERIAL AND METHODS

The studies were carried out on 5 Wistar male rats weighing 220-260 g. The animals were sacrificed by decapitation, after aether anaesthesia. Testes were removed and pieces of isolated seminal tubules were immersion fixed with 5% glutaraldehyde in 0.1 M phosphate buffer of pH 7.2 for 20 min. After fixation the specimens were washed in the same buffer and immersed in increasing concentrations of phosphate - buffered glycerol solutions (10,20 and 30% for 30 min. in each solution). They were then mounted on holders and rapidly frozen in propane cooled by liquid nitrogen. Freeze-fracturing was performed with an Edwards 306 apparatus at t=-110°C and P=5 x $(10)^{-7}$ Tr. The specimens were shadowed with platinium - carbon immediately after fracturing. Replicas were cleaned with 40% chromic acid for 1 hour and mounted on copper grids. They were examined using a Philips EM 300 electron microscope operating at 80 kV.

200 electron micrographs of replicas were obtained for analysis of mitochondria. Morphometric analysis was based on random sampling of seminiferous tubules. The morphological evaluations were performed using a IBAS 2000 picture analysing system. Analysis was performed on micrographs at x 81 000 magnification. In each type of germ cell, we measured the length of the edge of fracture plane deflection as related to the mitochondrium area. The obtained values were a certain length (L) per area ($\mu m/\mu m^2$). In order to perform an analysis which takes into consideration the different amount of smooth membrane fractures in the respective mitochondrial fractions, we determined on survey pictures the total area of smooth membrane fractures (Ms) and patchy membrane fractures (Mp). The single value L was then weighted by a factor of Mp/(Mp + Ms) for the particular preparation to obtained the Lp factor. The parameters in question are shown on Fig. 2. The significance of the differences between the values was tested by Kruskal - - Wallis rank sums test using IBM PC/XT personal computer.

RESULTS

Lengths of the fracture - plane edge during spermatogenesis are shown on Figure 3.

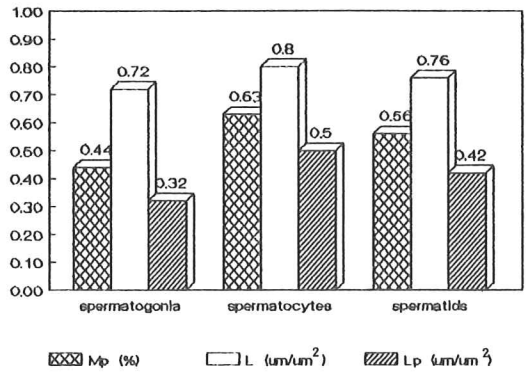

Figure 3. L is the mean value of the individual length measurements of the fracture plane deviations, Mp represents the relative area of mitochondrial patchy fracture faces and Lp is the weighted value with regard to the whole mitochondrial population.

Our results varied significantly in each group of germ cells. The length (L) of the fracture edge is highest in the group of spermatocytes at 0.8 ± 0.43 and decreases to 0.76 ± 0.39 in spermatids and to 0.73 ± 0.43 in spermatogonia. The proportion of mitochondria with patchy faces to those with smooth ones Mp (%) decreases from 63% in spermatocytes to 56% in spermatids and 44% in spermatogonia. It is evident that the parameter Lp obtained under the two former values is 0.5 in spermatocytes, 0.42 in spermatids and 0.32 in spermatogonia. It must be stressed that the presented values of the parameters were minimal in the mitochondria of spermatogonia in an orthodox steady state and increases statistically significantly in the condensing mitochondria of spermatocytes and less in spermatids.

DISCUSSION

In our study we applied the principles of Knoll and Brdiczka (1983). Our samples were prepared using glutaraldehyde fixation and glycerol cryoprotection. We analysed mitochondria "in situ" in germ cells. The results of Knoll and Brdiczka were based on the examination of cross - fractured isolated liver mitochondria. In our study the comparison of parameters Mp, L and Lp in different stages of spermatogenesis shows the highest values in the spermatocyte group. Based upon the data obtained in this study and upon results of earlier works using TEM, we demonstrate that the mitochondria reveal a characteristic feature ultrastructural configuration for the transitional states (state II and III) of high respiratory activity (Chance and Williams, 1956). Our results may differ from Knoll and Brdiczka's results as we applied glutaraldehyde fixation and glycerol cryoprotection. The differences in the two methods may influence the frequency of deflections possibly decreasing their amount after chemical fixation (glutaraldehyde). Our results indicate that the highest respiratory activity in mitochondria accompanied the meiotic process in spermatocytes. The spermatids also showed higher values than spermatogonia. Our data must be considered preliminary but our observations suggest that the transformation of the mitochondrial configuration and the metabolic states of mitochondria can be studied by the ultrastructure of freeze-fractured mitochondrial membranes during spermatogenesis and spermiogenesis.

REFERENCES

Andre J. Contribution á la connaissance du chondriome. Etude de ses modifications ultrastructures pentant la spermatogenése. J Ultrastruct Rec 1962;6/3:1-185.

Chance B, Williams G R. The respiratory chain and oxidative phosphorylation. Adv Enzymology 1956;17:65-134.

Cieciura L, Rydzyński K, Klitończyk W. Stereological studies on mitochondrial configuration in different organs of the rat. Cell Tiss Res 1979;196:347-360.

Cieciura L, Klimek I. Stereological studies on germ cell mitochondria of the rat during spermatogenesis and spermiogenesis. Folia Histochem Cytobiol 1988; 26/3: 177-184.

Fawcett DW. A comparative view of sperm ultrastructure. Biol Reprod Suppl 1970; 2: 90-127.

Hackenbrock CR. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. I. Reversible ultrastructural changes with changes in metabolic steady state in isolated liver mitochondria. J Cell Biol 1966;30:269-297.

Hackenbrock CR. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. II. Electron transport linked ultrastructural transformation in mitochondria. J Cell Biol 1968; 36: 345-369.

Hackenbrock CR, Rehn TG, Weinbach EC, Lemasters JJ. Oxidative phosphorylation and ultrastructural transformation in mitochondria in the intact ascites tumor cells. J Cell Biol 1971; 51: 123-137.

Knoll G, Brdiczka D. Changes in freeze - fractured mitochondrial membranes correlated to their energetic state. Dynamic interactions of the boundary membranes. Biochim Biophys Acta 1983; 733: 102-110.

Martino C, De Stefanini M, Floridi A, Marcanto ML, Agrestini A, Morelli M. Morphological and functional modifications of mitochondrion in the rat germ cells. J Submicrosc Cytol 1976; 6: 115-116.

Martino C, Floridi A, Marcanto ML, Malorni W, Bellocci M, Silvestrini B. Morphological, histological and biochemical studies on germ cell mitochondria of normal rats. Cell Tiss Res 1979; 196: 1-22.

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