ULTRASTRUCTURAL LOCALIZATION OF ATP-ASE ACTIVITY IN SUBCELLULAR FRACTIONS OF RABBIT BONE MARROW

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The ATPase activity in the various subcellular structures of the rabbit bone marrow was investigated by electron-histochemical and biochemical methods. Highest activity of the enzyme was found in the nuclear membranes, followed by the mitochondria, microsomes, and nuclei. On electron-microscopic investigation ATPase in the nuclei was found chiefly in the outer nuclear membrane. ATPase in the mitochondria is closely connected with the membranous structures, unlike in the mitochondria of other tissues. The enzyme in the microsomal fraction is located on the outer side of the microsomal vesicles.

Active differentiation of cells of the erythroid and myeloid series takes place in the bone marrow. The ATPase activity of the structures of these cells reflects to some degree their participation in energy generation.

Considering the high metabolic activity of the nuclei during differentiation, the ATPase activity of the nuclei and nuclear membranes is a subject of particular interest.

The object of this investigation was to study ATPase activity in isolated cell nuclei and other subcellular structures of the bone marrow. ATPase was studied by an electron-histochemical method and the enzyme was also determined biochemically in the isolated cell structures.

EXPERIMENTAL METHOD

Bone marrow cell nuclei were isolated from rabbits [4]. The mitochondria and microsomal fraction were isolated by the method of Schneider and Hogeboom [7].

Examination of films of the nuclei stained by the Romanovsky–Giemsa method in the light microscope showed that the preparation was contaminated with up to 5% of cytoplasm. Electron-microscopic investigation revealed nuclei with disturbances of the membrane as well as nuclear fragments (Fig. 1). Bone marrow nuclei are very fragile and are easily broken up by mechanical and chemical agents.

The isolated nuclei were suspended in 0.25 M sucrose and centrifuged for 10 min at 1,500 g. The residue of nuclei was treated with 4 ml of 0.25 M sucrose and irradiated with ultrasound (20 kHz) for 20-30 sec. The irradiated nuclear suspension was centrifuged for 7 min at 600 g. The supernatant, containing the membranous material, was mixed with two volumes of 69% sucrose, 2.7-ml samples of the resulting suspension were poured into centrifuge tubes, after which 0.3 ml layers of sucrose solutions with densities (at 20°C) of 1.22, 1.20, 1.19, 1.18, 1.16, and 1.14 were then successively added. The tubes and their contents were then centrifuged on the SW 39 bucket rotor of a Spinco L-50 ultracentrifuge at 36,000 rpm for 75 min (105,000 g). At the end of centrifuging the membranous material was concentrated as whitish bands between

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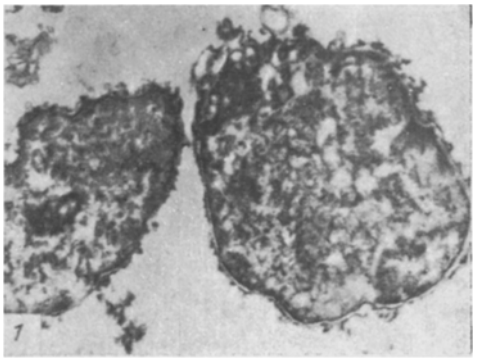

Fig. 1. Nuclei of rabbit bone marrow cells (Epon-812; 6000×).

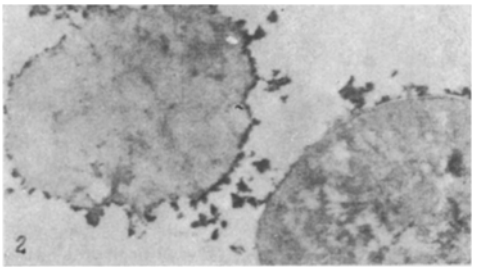

Fig. 2. Mg-activated ATPase activity in isolated rabbit bone marrow nuclei (38,000×).

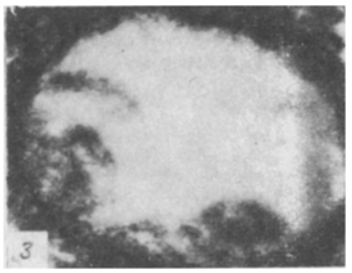

Fig. 3. Mg-activated ATPase activity in isolated mitochondria 120,000×).

the sucrose layers with densities of 1.22-1.20 and 1.16-1.18. Since there was very little material in each layer, the material of the two bands was combined, mixed together, diluted with distilled water in the ratio of 1 : 6, and centrifuged for 30 min at 10,000 g. The residue of membranes was used to prepare blocks for electron-microscopic investigation by standard methods [1]. Electron-microscopy showed that these preparations contained double membranes.

A thick suspension of the resulting fractions in 0.25 M sucrose was transferred by means of a pipette into an incubation medium containing 0.08 M Tris-maleate buffer (pH 7.4), 0.25 M sucrose, 2 millimoles magnesium acetate, 2 millimoles ATP, and 2 millimoles lead acetate. Unlike media with lead nitrate or with other buffer mixtures, a medium of this composition is stable and does not become cloudy if kept for many hours. After incubation for 30 min at 37°C the material was centrifuged and the residue resuspended in 2.5% glutaraldehyde in 0.05 M Tris-maleate buffer (pH 7.4) and 0.25 M sucrose. After fixation for 15 min in this medium the material was washed off with 0.25 M sucrose and postfixed in 1% OsO_4 solution in 0.1 M phosphate buffer (pH 7.2) with 0.25 M sucrose for 30 min. The material was then dehydrated and embedded in Epon 812, as described previously [1].

ATPase activity was determined by the method of Lohmann and Langen [5].

EXPERIMENTAL RESULTS

The ATPase activity in the nuclear fraction was 47 μmole PO_4/g protein/min at 37°C. The results of the electron-histochemical investigation (Fig. 2) showed that ATPase activity in the bone marrow cell nuclei is localized mainly in the outer nuclear membrane. However, where the outer nuclear membrane was damaged, a reaction for ATPase was also seen on the inner nuclear membrane. In nuclei evidently belonging to more highly differentiated blood cells the reaction product could be observed in the chromatin and nucleoli. The predominant localization of ATPase activity in the nuclear membrane was also confirmed by the fact that quantitative estimation of the activity of this enzyme in the fraction of nuclear membranes gave a figure of 223.8 μmoles PO_4/min at 37°C, several times higher than the activity of the whole nuclei.

Consequently, rabbit bone marrow nuclei do not differ from rat liver nuclei in their total ATPase activity [2]; however, the ATPase activity in the nuclear membranes is somewhat higher in the former than in the latter [2]. Electron-histochemically, ATPase was found chiefly in the outer nuclear membrane. The ATPase activity in the mitochondrial and microsomal fractions of the bone marrow cells was 191.4 and 117.7 μmoles PO_4/min respectively at 37°C. ATPase activity detected electron-histochemically in the mitochondria of the bone marrow cells is closely connected with the membranous structures, unlike the situation in the mitochondria of other, more highly differentiated tissues where the product of ATPase activity is found chiefly in the matrix [3, 6].

The mitochondria of undifferentiated bone marrow cells, like the nuclei, evidently possess low mechanical strength: during isolation the membranous components of the mitochondria are easily broken up, thereby facilitating access of the substrate to the enzyme. In the electron microscope swollen mitochondria, almost without cristae, can be found (Fig. 3). ATPase activity was found on the outer side of the mitochondrial membrane and in the remaining cristae.

The electron-histochemical investigation of the ATPase activity of the microsomal fraction showed that this enzyme is located on the outer side of the microsomal vesicles.

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