ISOLATION OF A MITOCHONDRIAL FRACTION FROM HUMAN BLOOD LYMPHOCYTES AND PLATELETS AND INVESTIGATION OF ITS OXIDATIVE PHOSPHORYLATION

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UDC 612.112.94+612.111.7].014.21:612.262.:612.398.145.1

Oxidative phosphorylation takes place in a mitochondrial fraction isolated from human blood lymphocytes and platelets. The method of isolation of the mitochondrial action from the platelets and lymphocytes and the results of its investigation in the electron microscope are described.

The object of this investigation was to determine oxidative phosphorylation in a mitochondrial fraction isolated from platelets and lymphocytes of healthy persons.

EXPERIMENTAL METHOD AND RESULTS

The isolation of mitochondria from lymphocytes and platelets requires a large number of cells (1×10^8 to 1×10^9) because of the few mitochondria which they contain.

Choice of the suspending medium and the pure fraction of lymphocytes from healthy human blood create difficulty because these cells have marked ability to aggregate; the best medium was found to be Krebs–Ringer bicarbonate buffer of the following composition (final concentration in millimoles): NaCl 120, KCl 29, KH_2PO_4 5.4, $NaHCO_3$ 127.

Lymphocytes considerably purified from contamination by granulocytes were obtained 18 h after taking the blood from the donors.* To purify the lymphocytes further from granulocytes, the cells were separated in columns (25×2.5 cm) with alternate layers of glass beads and cotton wool or polyacrylonitrile fiber; maximal volume 60 ml. After the column had been filled with the cell suspension, it was incubated at 37°C for 30 min. The suspension was then passed slowly (20 drops per minute) through the column, and rinsed out with autologous plasma, heated to 37°C, from which the platelets had previously been removed by centrifugation (1600 g, 30 min). While the suspension of leukocytes passed through the column, the granulocytes adhered to the beads and cotton wool, and the lymphocytes remained [3, 7]. The resulting suspension was centrifuged for 3 min at 228 g, the plasma was decanted, and the lymphocytes suspended in Krebs–Ringer bicarbonate buffer, pH 7.4. Isolation of the lymphocytes from donors' blood took about 24 h at 20°C. The viability of the lymphocytes, determined by supravital staining with trypan blue, was 90–96%. The purity of the lymphocyte suspension was 76–100%, and contamination was due to granulocytes; virtually no platelets were found in the suspension. Between 4×10^8 and 1×10^9 lymphocytes were isolated from one liter of blood.

Platelets were isolated by the method described earlier [1]. A 3.8% solution of sodium citrate in the ratio of 1:9 was used as the anticoagulant. Blood taken from the donors was allowed to stand for 18–20 h

*The writers are grateful to A. R. Maller for providing the fractions of donors' lymphocytes.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 6, pp. 45-47, June, 1973. Original article submitted July 10, 1972.

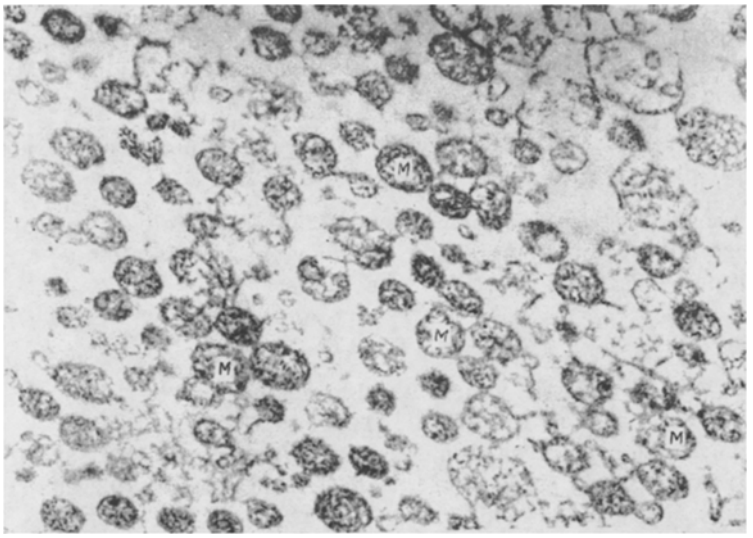

Fig. 1. Electron micrograph of mitochondrial fraction of lymphocytes. Isolated mitochondria with outer and inner (cristae) triple membranes can be seen (M). Magnification 30,000×.

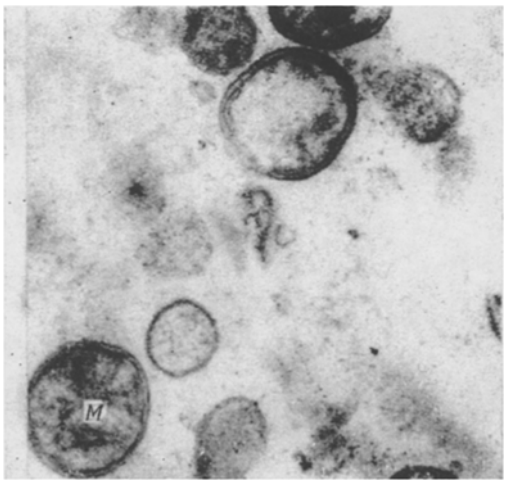

Fig. 2. Electron micrograph of granular fraction of platelets. Three-layered structure of outer and inner (cristae) membranes of mitochondria can be seen (M). Magnification 60,000×.

at 4-6°C. Plasma, containing platelets, was then separated from the erythrocytes and centrifuged at 2-4°C (1600 g, 30 min). To prevent agglutination of the cells, 0.3 ml of 5% neutralized EDTA solution was added to the residue of platelets and the cells were suspended in Krebs–Ringer bicarbonate buffer. The resulting homogeneous suspension of blood platelets was centrifuged for 2 min at 500 g to remove contaminating red cells.

EXPERIMENTAL RESULTS

The lymphocytes and platelets suspended in Krebs–Ringer bicarbonate buffer were broken up in ultrasound in a type MSE disintegrator at a frequency of 21 KH during cooling with a salt mixture for 20 sec. The degree of disintegration of the cells was verified morphologically in light and electron microscopes. The resulting homogenate was centrifuged at 2-4°C (3600 g, 10 min) to remove cell fragments (when working with lymphocytes and nuclei). The supernatant was centrifuged for 10 min at 80,000 g to isolate the mitochondrial fraction. The purity of the fraction and the structural integrity of the mitochondria were verified by examination of ultrathin sections in the electron microscope.

The residue containing the mitochondrial fraction of lymphocytes was fixed with OsO_4 [5], dehydrated with increasing concentrations of ethanol, and embedded in a methacrylate mixture. The residue of the mitochondrial fraction isolated from the platelets was fixed with glutaraldehyde and then in a buffered solution (pH 7.4) of 1% osmic acid, dehydrated, and embedded in Epon in the usual way. Ultrathin sections were cut on the LKB Ultratome and studied in the JEM-7 electron microscope.

The mitochondrial fraction isolated from lymphocytes contained no other cytoplasmic structures (Fig. 1). In the mitochondrial fraction isolated from the platelets, α-granules and vacuoles also were seen (Fig. 2), as was observed previously in a study of ultrathin sections of platelets [8] and also of the mitochondrial fraction [1]. Attempts to separate this fraction isolated from platelets into individual components have not yet led to the isolation of mitochondria uncontaminated by other cell organelles [9, 10].

The protein content was determined [4] in the resulting suspension of the mitochondrial fraction, and its oxidative phosphorylation was studied by an enzymic method [2, 6]. The reaction mixture (protein content 0.75-1.2 mg/ml) contained the following components (final concentrations in M): succinate 10, ADP 1.8, glucose 20, EDTA 0.3, and NAF 10; hexokinase 0.2 mg/ml.

After incubation for 30 min at 30°C, the quantity of glucose-6-phosphate formed was determined in the supernatant obtained after precipitation of the proteins with $HClO_4$.

The experiments showed that ATP is formed (about 200-500 μmoles/g mitochondrial protein) in the mitochondrial fraction isolated from human blood, lymphocytes, and platelets as a result of oxidative phosphorylation, a result not previously described for these cells.

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