SOME FUNCTIONAL CHARACTERISTICS OF DARK AND PALE CELLS

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Comparison of the structural features of the mitochondria in dark and pale cells with the results of a study of respiration of condensed, intermediate, and orthodox mitochondria showed that dark cells containing condensed mitochondria are in a resting state or a state of specific activity coupled with a high level of bioenergetic and biosynthetic processes. Pale cells, containing mainly intermediate mitochondria, are characterized by a state of active specific activity, accompanied by a high energy potential, increased utilization of energy, and a reduced level of biosynthetic processes. Pale cells containing predominantly orthodox mitochondria are characterized by a low level of energy metabolism and by predominance of destructive processes over processes of resynthesis of the cell structures.

KEY WORDS: dark and pale cells; morphofunctional types of mitochondria.

Dark and pale cells were first found under the optical microscope at the end of the last century [10]. At that time attempts were made to interpret their function and contradictory views were expressed on this problem. In particular, dark cells were regarded by some authors as cells in a state of rest [8], by others as functionally active cells [1]. The problem has not been solved by the introduction of electron-microscopic techniques and further morphofunctional investigations are necessary at the intracellular level. Comparison of the results of autoradiography with data on ultrastructure has led to the conclusion that dark cells are in a resting state, whereas pale cells are in a state of specific activity [3, 4].

However, for a final solution to the problem of the functional activity of these cells, the characteristics of their energy metabolism must be settled.

EXPERIMENTAL METHOD

Several tissues were investigated in various pathological states: tissues of the heart, liver, and kidneys of dogs during a 9-h chronic compression syndrome (CCS) and decompression for 2 h (15 dogs), human skeletal muscles during electrical stimulation (three cases), and on the 13th day of CCS (four cases), skeletal muscles of dogs during ischemia for 6 h (five animals), and on the 5th-7th day after subsequent regrafting of a limb (five dogs).

The main method of investigation was to compare the structure of the mitochondria in the dark and pale cells with the results of function tests on different types of these organelles [2].

Material for electron microscopy was fixed in osmium tetroxide and treated in the usual way. Sections were examined in the JEM-100V electron microscope.

EXPERIMENTAL RESULTS

A characteristic feature of the structure of the mitochondria is their polymorphism, which is clearly marked under pathological conditions even within the same cell. The results of this investigation showed

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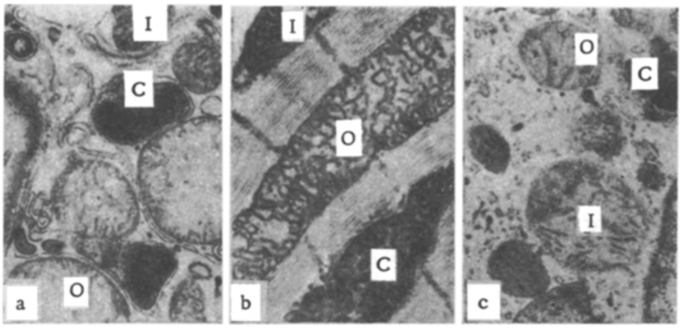

Fig. 1. Different types of mitochondria: a) Distal tubule of kidney of dog with CCS (18,000 ×); b) myocardium of left ventricle of dog with CCS (15,000 ×); c) liver of dog with CCS (20,000 ×). Here and in Figs. 2 and 3, C, I, O indicate condensed, intermediate, and orthodox mitochondria, respectively.

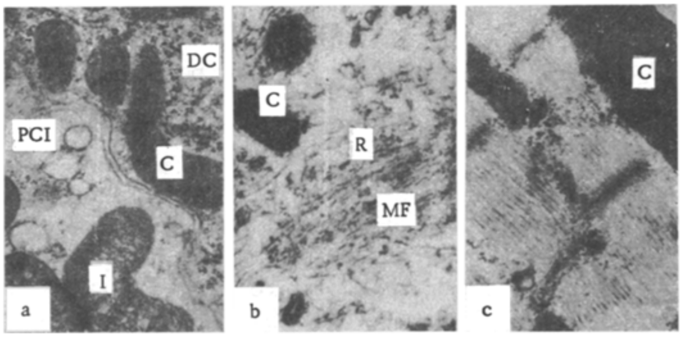

Fig. 2. Predominance of condensed mitochondria in dark cells: a) Distal tubule of kidney of dog with CCS: Dark cell lies next to pale cell containing intermediate mitochondria (15,000 ×); b) myoblast in focus of regeneration of skeletal muscle during CCS (20,000 ×); c) skeletal muscle of dog on 7th day after regrafting of limb after ischemia for 6 h (18,000 ×). DC) Dark cell; PCI) pale cell with intermediate mitochondria; R) ribosomes; MF) myofibrils.

that, irrespective of the type of tissue, all forms of mitochondria observed could be divided conventionally, in accordance with Beketova's classification [2], into three types: condensed, intermediate, and orthodox (Fig. 1). Condensed mitochondria are characterized as a rule by an elongated or irregular shape, an electron-dense granular matrix, and numerous tightly packed cristae with a widened intermembranous space. Intermediate mitochondria differ from the first group by being rounder and having a somewhat more translucent and finely granular matrix. The number of cristae in them and in the condensed mitochondria was comparable, but the space inside the cristae was relatively narrow. Orthodox mitochondria were usually round in shape with a greatly swollen electron-transparent matrix and reduced cristae. The rates of respiration for these three types of mitochondria from liver tissue were determined in different metabolic states, together with the respiratory control and the coefficient of phosphorylation ADP/O [2]. Considering these functional pointers to the predominance of one type of mitochondria in the dark or pale cells [6], the function of the cells could be assessed from the state of their mitochondria.

The ultrastructure of the dark cells observed in the tissues studied (Fig. 2) agreed mainly with that described by Sarkisov [6]. Their characteristic feature was that their mitochondria were predominantly of the condensed type. High rates of respiration and coupled oxidative phosphorylation are observed with these organelles [2], reflecting their high energy potential. Condensed mitochondria are also characterized by high respiratory control on endogenous substrates and positive values of the difference $V_4 - V_2$ (the rates of oxygen utilization on succinate after and before phosphorylation, respectively), evidence of maximal accumulation of reserves of high-energy phosphates in them. The latter, together with the high energy potential of these organelles, is regarded [9] as an essential condition for well-marked biosynthetic processes.

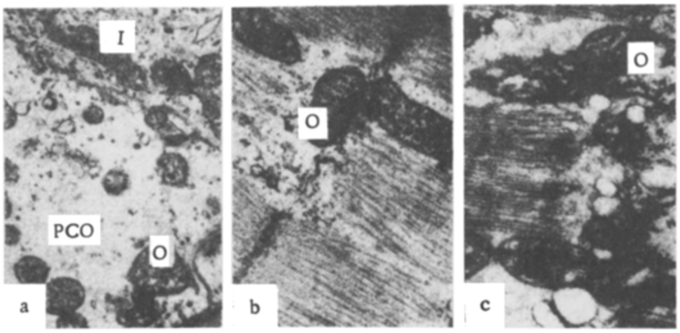

Fig. 3. Pale cells containing orthodox mitochondria: a) Collecting tubule of kidney of dog with CCS: pale cell with orthodox mitochondria lie next to pale cell containing mitochondria of intermediate type (10,000 ×); b) skeletal muscle of dog during ischemia for 6 h (20,000 ×); c) human skeletal muscle after electrical stimulation (20,000 ×). PCO) Pale cells with orthodox mitochondria.

These results of the investigation of mitochondrial respiration show conclusively that dark cells are in a state of rest and accumulation of substrates. In the writers' view, the high biosynthetic potential of condensed mitochondria reflects the predominant concern of dark cells with the formation, renewal, and regeneration of intracellular structures, or with synthetic processes connected with their specific activity. Some dark cells are in fact cells from foci of regeneration, and these have been shown to have a high level of DNA synthesis [4]. The writers' investigations showed that myoblasts from regenerating muscle tissue, which contain single myofibrils, chains of ribosomes forming them, condensed mitochondria, and so on (Fig. 2b), are also dark cells. In addition, because of the predominance of condensed mitochondria, muscle fibers at the 5th–7th day of regrafting a limb in dogs after ischemia for 6 h are also regarded as dark cells of a special type, in a state of renewal of the intracellular structures (Fig. 2c). Dark cells whose specific activity requires a high level of synthetic processes and of energy metabolism have been found in the mucous membrane of the stomach [7] and in other tissues.

Pale cells, described by Sarkisov [6], along with other typical features are characterized by mitochondira of intermediate type (Fig. 2a). Such mitochondria were found to have the highest efficiency of oxidative phosphorylation, low respiratory control on endogenous substrates, and a negative value of the difference $V_4 - V_2$. These data, indicating a high energy potential of the organelles and some depletion of their reserves of high-energy compounds, suggest that the pale cells with intermediate mitochondria are cells in a state of specific activity. The increased expenditure of energy with the switch of the cells into a state of active specific activity [5] is not accompanied by any further significant expenditure of structures. Their cytoplasm still has a high content of cytogranules and in the electron microscope it appears only a little paler than the cytoplasm of the dark cells.

Together with pale cells containing mainly intermediate mitochondria, other pale cells deficient in cytogranules, frequently vacuolated, and containing mitochondria of orthodox type also were observed (Fig. 3). Swelling of the matrix of these organelles and destruction of their cristae were accompanied by a decrease in the level of respiration and uncoupling of oxidative phosphorylation. The predominance of orthodox mitochondria in pale cells, in muscle fibers after ischemia for 6 h (Fig. 3b), for example, and also during intensive activity of the muscle fibers as a result of electrical stimulation (Fig. 3c), is evidence of predominance of the breakdown of structures in these cells over processes of their resynthesis.

The study of morphofunctional types of mitochondria can thus help to identify the level of bioenergetic and biosynthetic processes in dark and light cells and can thus shed some light on the mechanisms of adaptation of an organ to different conditions of function.

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