CHAPTER 14

Properties of Mitochondria

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A. Introduction
Recently it has become evident that antitumor drug resistance is associated with some features of target enzymes and those enzyme systems which participate in drug transport, activation and catabolism (BROCKMAN 1974; BELOUSOVA 1978; BELOUSOVA and GERASIMOVA 1980).

Among the effective antitumor drugs used in the management of clinical cancers, alkylating agents are still of primary importance (ZUBROD 1972; CARTER and SLAVIK 1977). General features of these cytostatics include their ability to alkylate cellular DNA, RNA and proteins, to form DNA-DNA and DNA-protein cross-links, and to induce the formation of single-strand and double-strand breaks in DNA molecules (LAWLEY and BROOKES 1967; ROSS 1962; ROSS et al. 1978).

Damage to cellular genetic material leads, in turn, to disturbances in DNA replication, transcription and protein synthesis, and to lethal and mutagenic effects (LOVELESS 1966; MITSKEVITSH et al. 1972; KLAMERTH 1973; VEROVSKY and GORBATCHEVA 1979).

The initial degree of DNA alkylation may be the same in cells sensitive and resistant to cytostatics, but in resistant cells defects in DNA structure are effectively eliminated by repair enzymes. Therefore the presence of a potent enzyme system for DNA repair is generally accepted as an appropriate criterion of cell resistance to alkylating agents (ROBERTS 1980).

However, cytotoxic effects of antitumor alkylating agents are not limited to damage to the structure and template functions of DNA and chromatin alone.

B. Damage of Mitochondrial Membranes by Alkylating Agents
It was first shown by BELOUSOVA and colleagues (BELOUSOVA 1965; BELOUSOVA et al. 1964, 1966) that some chloroethylamines with aromatic and more complex carriers (sarcologsine, its dipeptides, and other analogs) are able to uncouple respiration and oxidative phosphorylation in isolated mitochondria of tumor and normal cells.

The detailed study of these effects by ROMANOVA (1971, 1972) suggested that sarcologsine is similar to classic uncouplers of the dinitrophenol type. The uncoupling effect is manifested by (a) the inhibition of ATP synthesis, (b) activation of the latent mitochondrial ATPase and (c) releasing of the respiratory control. Like 2,4-dinitrophenol (DNP), sarcologsine uncouples respiration and phosphorylation at all three points. Similar effects were also shown for chlorophenacyl (GUDZ et al. 1974; YAGUZHINSKY et al. 1976).
The dipeptides of sarcolysine are more potent inhibitors of oxidative phosphorylation than the parental compound. They exhibit a rather strong inhibitory effect on respiration, being in this respect similar to oligomycin (Romanova 1972; Belousova 1978).

It was found that tumor mitochondria are much more sensitive to the uncoupling action of alkylating agents than those of the liver (Belousova et al. 1964; Belousova and Romanova 1971).

During the investigation of in vivo effects of sarcolysine and its dipeptides on oxidative phosphorylation in mitochondria of animal tumors and normal tissues, a correlation between energetics impairment and cytotoxicity was shown (Spaskaja et al. 1968; Romanova and Sofina 1969; Belousova and Romanova 1971).

Among normal tissue spleen and thymus were the most vulnerable to cytostatics, while liver was much less sensitive. The uncoupling effect of cytostatics was maximal in thymus and spleen mitochondria and minimal in those of the liver.

Tumor mitochondria were highly sensitive to uncoupling effects of alkylating agents. Thus in these investigations a positive correlation between uncoupling effects and cytotoxicity of antitumor alkylating agents was found.

C. The Structure and Functions of Energy-Coupling Complexes in Mitochondria

The enzymes of respiration and oxidative phosphorylation are known to be built up into the inner membrane of mitochondria as repeated multienzyme complexes (Green 1974) (Fig. 1). Each complex contains, in its basal part, three units of the respiratory electron transfer chain and a transhydrogenase. At the inner side of this complex there is an energy-transforming unit, which, in turn, is composed of two subunits: the headpiece (factor $F_1$) and the basepiece (factor $F_0$). Both subunits form an ATPase complex, which accomplishes the transformation of substrate oxidation energy into the high-energy phosphoanhydride bond of ATP.

The molecular mechanism of coupling is not yet understood. Several different hypotheses exist concerning the nature of the primary intermediate which acquires energy directly from the substrate oxidation and which is used as an energy donor for ATP synthesis (Racker 1976).

The “chemical” hypothesis (Slater 1966; Racker 1976) suggests the hypothetical unphosphorylated high-energy compound $X \sim I$ as the first intermediate of energy transformation.

In the “conformational” hypothesis it is assumed that the transformation of oxidoreductive energy occurs in some energized conformations of membraneous proteins (Green 1974; Boyer 1975; Gomez-Puyou et al. 1978). The “chemio-osmotic” hypothesis of Mitchell (1977) postulates the establishment of an electro-chemical potential gradient of protons and transmembrane potential during substrate oxidation as a direct mechanism of ATP synthesis.

But whatever the mechanism of coupling of biological oxidation energy with ATP synthesis consists of, experimental data suggest that in intact mitochondria the multienzyme complex involved in this process is working as ATP synthetase.