RESPIRATION AND STRUCTURAL CHANGES IN MITOCHONDRIA OF THE INTESTINAL EPITHELIAL CELLS DURING ABSORPTION OF AMINO ACIDS IN IRRADIATED ANIMALS

A. V. Strelina, T. D. Batyuto, and A. A. Manina

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Experiments on rats showed stimulation of respiration and ultrastructural transformations of the mitochondria in jejunal epithelial cells during absorption of amino acids (a mixture of alanine and glycine) in intact animals. However, 30 min after whole-body-x-ray irradiation in a dose of 600 rad the absorption of amino acids was accompanied by a decrease in the oxygen utilization level and by weakening of the reaction of the ultrastructures. The results are discussed from the standpoint of the effect of radiation on the energy production system and on energy-dependent functions.

Many workers have studied the effect of radiation on tissue respiration in various organs [1-3, 7]. In an investigation of the intestine, Strelina [4, 6] showed changes in the activity of certain enzymes in the terminal stage of aerobic oxidation, indicating a disturbance of energy production. It is of great importance to determine the role of these disturbances in physiological functions connected with expenditure of energy. A characteristically energy-dependent function in the intestine is the absorption of amino acids. Jasper and Bronk [8, 11] showed that incubation of the epithelium of the intestinal mucosa in medium containing amino acids is accompanied by increased oxygen utilization. Meanwhile, other workers have shown that processes consuming the energy of the cell are accompanied, in particular, by characteristic changes in the ultrastructure of the mitochondria [10, 12, 13]. Some of them [10, 12] associate the transduction of energy generated during electron transport directly with mechanical changes in the structure of the mitochondria, consisting of conformational transformations of the inner compartment. An energized and a nonenergized configuration of the mitochondria are accordingly distinguished.

The object of the present investigation was to make a combined study of respiration and the ultrastructural characteristics of the epithelial cells of the intestinal mucosa of irradiated animals during the absorption of amino acids.

EXPERIMENTAL METHOD

Albino rats weighing 180-200 g were used. The rats received the ordinary animal house diet. Whole-body x-ray irradiation was given in a dose of 600 rad on the RUM-3 apparatus (voltage 180 kV, distance from anode 40 cm, filters 0.5 mm Cu and 1 mm Al, dose rate 44 rad/min). The absorption of O_2 by a scraping from the jejunal mucosa was determined by Warburg's method in microatoms per 10 mg protein. Protein was determined by Lowry's method. The incubation medium was of the following composition: 118 mM NaCl, 25 mM $NaHCO_3$, 4.2 mM KCl, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2 \cdot 6H_2O$; pH 7.5.

A mixture of alanine and glycine (molar ratio 0.6 : 1.0) in a concentration of 1 g/ml was used in the experiments to study absorption of amino acids.

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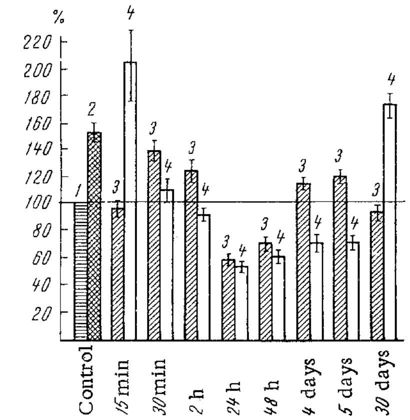

Fig. 1. Respiration of jejunal epithelium of rats without amino acids and during absorption of a mixture of alanine and glycine after whole-body irradiation of the animals in a dose of 600 rad: 1) control; 2) control + amino acids; 3) irradiation; 4) irradiation + amino acids. Abscissa, time after irradiation; ordinate, level of respiration (in percent; respiration in control animals without addition of amino acids taken as 100%).

In the experiments to investigate the ultrastructures the rats were lightly anesthetized with ether, a segment of jejunum 15 cm in length was separated by ligatures, and 1.5 ml of incubation medium with or without the addition of the amino-acid mixture was injected into it from a syringe, and incubation was allowed to continue for 15 min. The villi were fixed in 5.5% glutaraldehyde solution in cacodylate buffer, pH 7.2, at 4°C and then postfixed in 1% OsO_4 solution and embedded in Epon. Sections were cut on and LKB ultratome and photographed in a Hitachi electron microscope. The ultrastructural characteristics were studied 30 min and 2 h after irradiation, and respiration 15-30 min, 2, 24, and 48 h, and 4, 5, and 30 days after irradiation.

EXPERIMENTAL RESULTS

Absorption of O_2 from the epithelium of the jejunal mucosa during incubation without amino acids and with the addition of a mixture of alanine and glycine in intact animals and at various times after whole-body x-ray irradiation in a dose of 600 rad is shown in Fig. 1.

If the level of respiration of control animals in the absence of amino acids is taken as 100% it will be clear that the absorption of amino acids, accompanied by stimulation of O_2 absorption, reaches a level of 153%. In these animals, the overwhelming majority of mitochondria in the epithelial cell of a jejunal villus (Fig. 2a) incubated without the addition of amino acids were in a nonenergized or "orthodox" configuration, corresponding to the 4th metabolic state of Chance and Williams [9, 12]. In this state the mitochondria have clearly defined outer and inner membranes, clearly arranged cristae, and a uniformly pale matrix. During absorption of amino acids (Fig. 2b) the mitochondria undergo very sharp changes which affect predominantly the inner compartment. The cristae become condensed and lose their regular arrangement, and the matrix becomes optically dense and is in close contact with the inner membrane of the cristae. Sharply defined pale intercristal spaces appear. These mitochondria are generally called condensed or energized, and they are characteristic of the 3rd metabolic state of Chance and Williams.

In the control animals the absorption of amino acids was accompanied by simultaneous stimulation of respiration and the appearance of a condensed state of the mitochondria.

The same pattern as regards O_2 absorption was still preserved 15 min after irradiation, i.e., respiration during incubation without amino acids corresponded to the control level and was considerably stimulated in the presence of amino acids.

The situation was radically changed 30 min and 2 h after irradiation. Without amino acids a slight increase in the intensity of respiration was observed (up to 142 and 123% for 30 min and 2 h respectively), but in the presence of amino acids not only was respiration not increased, but it was significantly inhibited. This is presumably evidence of injury to the mechanisms of energy accumulation at these periods after irradiation. Other evidence in support of this view is given by the character of the ultrastructural transformations of the mitochondria. The mitochondria 30 min after irradiation were very swollen, their cristae were partly destroyed, and their matrix was translucent. At the same time, in normally phosphorylating mitochondria there is a characteristic dynamic oscillation of swelling $\rightleftharpoons$ contraction. During absorption of amino acids in this period after irradiation only some mitochondria acquired the condensed configuration, but they did so to a far lesser degree, which evidently corresponds to a different stage of condensation; in particular, the regular orientation of the cristae was not lost.

The changes in the mitochondria continued to progress 2 h after irradiation (Fig. 2c). These were swollen even more, and were ovoid or spherical in shape (normally most mitochondria are long and rod-shaped), frequently the outer membrane was ruptured, the cristae were largely destroyed, and vacuoles appeared. However, even at this time after irradiation,better preserved mitochondria or even parts of mito-

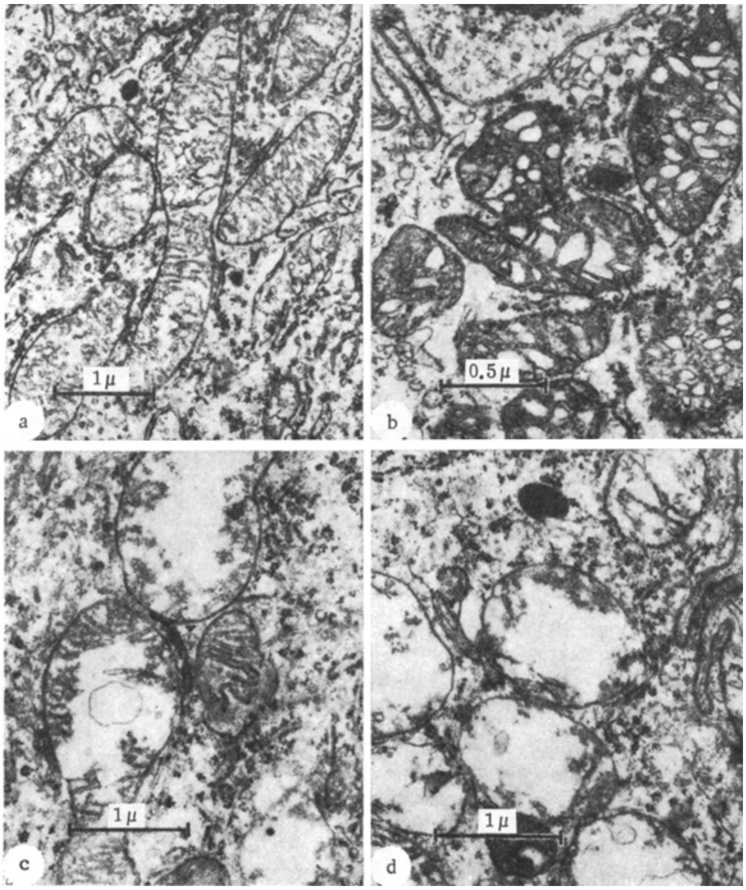

Fig. 2. Mitochondria of epithelial cells of villi from control animals (a and b) and 2 h after irradiation (c and d). a and b) Without addition of amino acids; c and d) with addition of a mixture of alanine and glycine.

chondria were still found, the other parts being swollen and containing vacuoles. The condensed configuration of the cristae was not observed 2 h after irradiation in the presence of amino acids (Fig. 2d).

A new property of respiration was observed 24 and 48 h after irradiation. Absorption of O_2 with amino acids was considerably reduced (58 and 65% of the control level respectively) 24 and 48 h after irradiation and in the presence of amino acids it continued at approximately the same level (56 and 61% respectively). The decrease in the intensity of respiration at these times after irradiation cannot be attributed unequivocally to a decrease in the cell population in the crypts and the villi of the intestine, for the O_2 absorption was calculated with respect to a constant (10 mg) protein content of the mucosa. In addition, as Strelina [6] found in experiments at the same times and with the same dose of irradiation, inhibition of the activity of the final enzyme of the tissue respiration cycle (cytochrome oxidase) is observed in the cells of the jejunal villi in mice.

Recovery of respiration was observed in the medium without amino acids four and five days after irradiation (116 and 119% respectively), but it is important to stress that in the presence of amino acids the level of respiration was lowered (69 and 70% of normal). Recovery of the original characteristic pattern of the intact animals could not be accepted as having taken place until 30 days after irradiation. At this time respiration without amino acids had reached 90% of the control level, compared with 175% in the presence of amino acids.

These experimental results show a definite correlation between the metabolic activity and ultrastructure of the cell affecting above all the inner membrane of the mitochondria, where the systems of enzymes responsible for energy production are located.

Whole-body x-ray irradiation injures the tissue respiration system in the epithelium of the jejunal mucosa and distorts the energy-dependent process of amino-acid absorption. The systems coupling the energy of electron transport to the energy of the chemical bonds are evidently more sensitive.

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