EFFECT OF RADIATION SICKNESS AND RADIOPROTECTIVE ACTION OF CYANIDE ON CYTOCHROME OXIDASE ACTIVITY IN EPITHELIUM OF JEJUNAL VILLI IN MICE

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Whole-body x-ray irradiation of mice in a dose of 600 R ($LD_{65/30}$) inhibits cytochrome oxidase activity in the epithelium of the jejunal villi. Although administration of sodium cyanide, a cytochrome oxidase inhibitor, before irradiation causes a sharp initial depression of enzyme activity, this is followed by a more rapid recovery. This indicates the cyanide has a protective action on the cytochrome system against radiation damage. The survival rate under these circumstances is increased by 40%.

An essential biochemical manifestation of the action of ionizing radiation on the animal organism is uncoupling of oxidation from phosphorylation, and this is necessarily accompanied by disturbance of the energy balance in the irradiated organism and, consequently, by a shift from normal metabolism toward increased hydrolysis and depression of synthesis. The problem of which reactions or systems are primarily responsible for the uncoupling effect is at present being actively discussed.

A question of particular interest in this field is the study of the activity of cytochrome oxidase (COase), an enzyme whose place is at the end of the tissue respiration chain, and which thus reflects the state and integral relationships of the basic energy-producing systems.

In an early series of investigations the writer demonstrated the sensitivity of COase to irradiation, observable within a few hours after whole-body irradiation of animals in doses of 2000-4000 R.

There is little information in the literature concerning the state of energy metabolism in the later stages of development of radiation sickness. However, there are indications that recovery after irradiation is dependent on the state of energy metabolism [3, 10, 11].

The object of the present investigation was to study the dynamics of COase activity and to compare it with the dynamics of mortality among the animals from radiation sickness. In addition, to assess the possible importance of changes in activity of this enzyme in the course of radiation sickness, the character of this change and of mortality among the animals was studied after administration of sodium cyanide, a radioprotective substance which is also a specific inhibitor of COase.

EXPERIMENTAL METHOD

Experiments were carried out on 300 noninbred albino mice weighing 19-22 g. Conditions of irradiation: RUM-3 apparatus, 180 kV, 15 mA, distance from anode 30 cm, filters 0.5 mm Cu and 1 mm Al, dose rate 76 R/min.

A modified photometric method was developed suitable for histochemical investigation of oxidative enzymes. COase activity was determined by Burstone's method [7], using n-phenyl-p-phenylenediamine and 1-hydroxynaphthoic acid.

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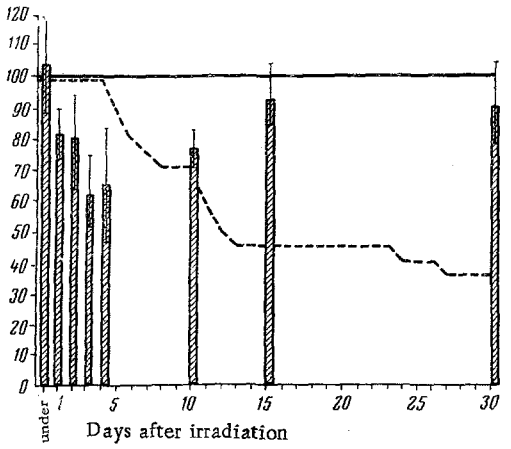

Fig. 1. Survival rate of mice following irradiation in dose of 600 R. Abscissa, days after irradiation; ordinate, percentage of surviving animals and COase activity (in percent of control level). Continuous line, percentage of surviving animals in control; broken line, percentage of surviving irradiated animals; columns denote COase activity in jejunum of irradiated animals.

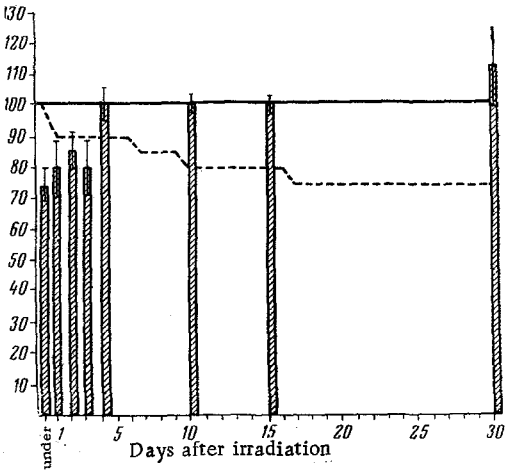

Fig. 2. Survival rate of mice and COase activity in epithelial cells of jejunal villi after irradiation in a dose of 600 R under the radioprotective acting of sodium cyanide. Legend as in Fig. 1.

The animals were decapitated without anesthesia. Pieces of small intestine were frozen with liquid nitrogen.

Two pieces of tissue (from control and experimental animals) were frozen simultaneously on the stage of a rotary microtome in a cryostat, and sections were cut to a thickness of 20 μ. Incubation and subsequent treatment of the sections were carried out on slides. The photomicrographs of the sections were made with the aid of filters allowing maximal transmission of the color of the dye. A step wedge was fitted into the cassette of the camera. Photometric examination of the negatives was carried out by means of the MF-4 microphotometer. The results were expressed in conventional units of optical density and were analyzed by statistical methods. Enzyme activity was estimated in the epithelial cells of the jejunal villi.

EXPERIMENTAL RESULTS

The survival rate and dynamic of COase activity in the epithelial cells of the jejunal villi of mice irradiated in a dose of 600 R are given in Fig. 1.

After irradiation in a dose of 600 R the animals began to die on the 4th day, and this first peak of mortality ("intestinal death") lasted 8 days. The second wave of mortality ("bone-marrow death") corresponded to the 11th-14th days, after which the curve of mortality of the animals followed a smoother course until the end of the period of investigation ($LD_{65/30}$).

COase activity in the epithelial cells of the jejunal villi was studied at various times: until 1 h, at 24-48-72 h, and 4-10-15-30 days after irradiation. The first significant decrease in COase activity was recorded 24 h after irradiation (inhibition 20%). The greatest depression of enzyme activity was observed after 3-4 days (inhibition reached 38-34%, respectively), after which activity began to increase, although it remained significantly lower for 10 days after irradiation. After 14 days it had returned to its initial level.

Comparison of the results of this series of experiments suggests a correlation between survival rate and level of enzyme activity in the irradiated animals. Mortality ("intestinal death") begins at the moment of greatest depression of activity of the energy-producing system in the jejunum (3-4 days). The peak of intestinal death continues during a period characterized by a low level of activity of this system. The recovery period is accompanied by normalization of enzyme activity.

It is suggested that the energy deficiency due to disturbance of tissue respiration in the epithelial cells of the villi after irradiation may manifest itself as inhibition of synthesis, and in particular, it may inhibit the synthesis of invertase [2], and also other processes linked with the utilization of energy, such as the absorption and transport of glucose [1].

This would explain the correlation between the state of activity of COase, reflecting integral relationships between energy-producing systems in the intestine, and the severity of the subsequent lesions causing "intestinal death."

In the next series of experiments the survival rate and dynamics of activity of COase in epithelial cells of the jejunal villi were studied in mice irradiated with a dose of 600 R and receiving the radioprotective agent cyanide.

Sodium cyanide, in a concentration of 5 μg/g body weight, was injected intraperitoneally immediately before irradiation. These results are given in Fig. 2.

As a result of the radioprotective action of cyanide, 10% of the animals (of the 25% dying throughout the whole period of investigation) died on the 1st day after irradiation. Both peaks – "intestinal" and "bone-marrow" death – were considerably flattened; after the 17th day after irradiation no more animals died. The radioprotection afforded by cyanide was 40%. The curves of survival in animals protected and unprotected by cyanide thus differed sharply. There was a corresponding difference in the dynamics of changes in COase activity in the protected and unprotected animals. The most important factor in radiation protection was the initial inhibition of COase, due to the action of the poison, and coinciding with the time of irradiation (inhibition by 27%), followed by a relatively rapid recovery during the 4 days after irradiation; the COase level did not fall further throughout the remainder of the period of investigation. There are many reports in the literature of investigations into the mechanisms of the radioprotective action of cyanide. The most widely held view is that the protective action of cyanide consists essentially of a vasoconstrictor effect, accompanied by hypoxia of the blood supplying the tissues. However, this hypothesis by no means gives a complete explanation of the many different effects of cyanide, and it cannot interpret many of the facts. For example, during the protective action of cyanide the O_2 tension in the brain tissues is not lowered; in vitro, cyanide protects systems in which the O_2 tension is unchanged [5, 6].

Many workers consider that the most important biochemical effect of cyanide is reversible inactivation of COase as a result of the formation of a COase – CN complex which cannot transfer atmospheric O_2 to electron carriers. In this state, cytochrome oxidase displaces the equilibrium of electron carriers toward reduction, and thus helps to increase their radioresistance [9]. This point of view is in agreement with the results of the present experiments. As a result of the formation of a COase – CN complex, COase activity is sharply reduced at the time of irradiation, but later, with dissociation of this complex, COase activity increases and contributes to the provision of energy for reduction processes.

Certain other compounds forming complexes with COase, such as cysteine [8], may also have a radioprotective action similar to that of cyanide.

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