HISTOCHEMICAL INVESTIGATION OF THE SUCCINATE DEHYDROGENASE ACTIVITY IN THE SMALL INTESTINE OF ANIMALS EXPOSED TO IONIZING RADIATION

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Evidence is steadily being accumulated showing that tissue respiration is disturbed during the action of ionizing radiation. It has been found that the disturbance of oxidative phosphorylation caused by radiant energy is associated with inhibition of the electron transport system [6-11]. The idea is developing [3, 4] that the aerobic link in the chain of tissue respiration is vulnerable because of the effect of ionizing radiation on the metal-containing biochemical systems, including the oxidative enzymes.

The facts and hypotheses described above draw attention to the investigation of the state of the enzyme systems of oxidative metabolism during the action of radiation.

In the present investigation the activity of one of the enzymes of the oxidative cycle – succinate dehydrogenase – was studied during the action of ionizing radiation.

Most investigators consider that succinate dehydrogenase is a radioresistant system [1, 7, 10]. However, reports have been given of changes regularly taking place in the activity of this enzyme in radiation sickness [2, 5].

In biochemical investigations, during treatment of the material structural changes may take place in the mitochondria, leading to dissociation of enzyme and substrate and also to their contamination by nuclear or cytoplasmic substances which may have a protective or inhibitory action on the enzyme. With this in mind, the histochemical method of investigation was chosen, in which the structure of the mitochondria is preserved and the reaction is observed in situ.

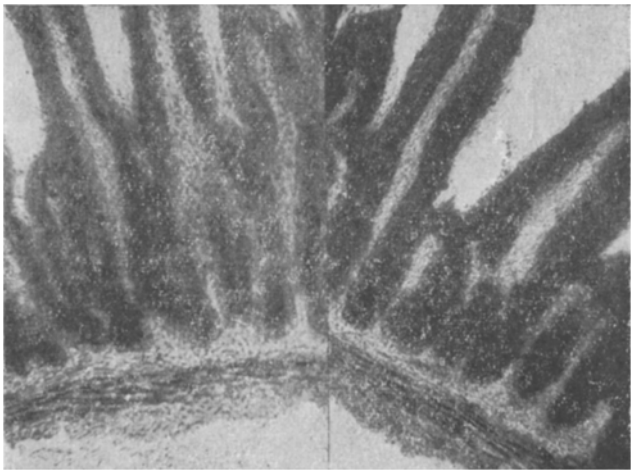

Fig. 1. Deposition of formazan in sections of the small intestine of control and experimental mice. Dose 2000 R, 2 h after irradiation. On the left) control, on the right) experiment. The areas of intensive deposition of formazan correspond to +++. 140×.

Laboratory of Biochemistry, Central Roentgeno-Radiological Research Institute, Ministry of Health of the USSR, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR, N. A. Kraevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 2, pp. 113-117, February, 1967. Original article submitted May 24, 1965.

Effect of Whole-Body X-Ray Irradiation on Oxidation of Succinate in Sections of Small Intestine

Difference in intensity of staining of sections obtained from control animals (comparison between pairs)		Dose of radiation (in R)	Activation of reaction by irradiation (difference in staining between control and experiment) Time after irradiation: immediate	1 h	2 h	4 h	6 h	24 h	48 h	72 h
Mice	0 0 0 0 0	600 (Mice)	0 0 0 0 0 0 0 0 +	0 0 0 0 + ++ ++	0 0 0 + + + ++ ++ ++	0 + + + + ++ ++	0 0 0 + + + ++	0 0 0 0 0 0 +		
	0 0 + +	2 000 (Mice)	0 0 0 0 0 +	0 0 + + + ++ ++	0 + ++ ++ +++ ++++ ++++			0 0 + ++ ++ +		
		4 000 (Mice	0 0 0 0 0							* + + + ++
Rats	0 0 0 0 0 0 0 +	4 000 (Rats)	0 0 0 0 0 + +	0 + + ++ ++ ++ +++	0 0 + + + ++ ++			+* + + + + + + ++	+* + ++ ++ ++ ++	+* + + ++ ++ ++ ++

*Assessment of reaction of muscle layers.

EXPERIMENTAL METHOD

Experiments were carried out on 200 noninbred albino mice weighing 19-22 g and on 100 noninbred albino rats weighing 160-180 g.

The small intestine was investigated, because the various structural elements of the intestinal wall differ greatly in their radiosensitivity. It therefore presents specific possibilities for analysis of the action of radiation on the enzyme.

Irradiation was given in doses of 600, 2000, and 4000 R. The animals were sacrificed immediately, and 5-10 min and 1, 2, 4, 6, 48, and 72 h after whole-body x-ray irradiation. The conditions of irradiation were: RUM-3 apparatus, voltage 180 kV, current 15 mA, distance from anode 30 cm, filters 0.5 mm Cu + 1 mm Al, dose rate 76-86

The investigations were carried out by the method of Nachlas and co-workers [8], using nitro-BT, which receives electrons in the electron-transport chain at the level of succinate dehydrogenase – cytochrome b [9]. During oxidation of succinate, the colorless tetrazolium salt is reduced and converted into the colored formazan.

After decapitation a piece of the small intestine was quickly excised from the experimental animals and placed in a freezing microtome. Sections cut to a thickness of 15-20 μ were transferred to an incubation medium in which there were kept at 37° for 15 min. The stained sections, after suitable treatment

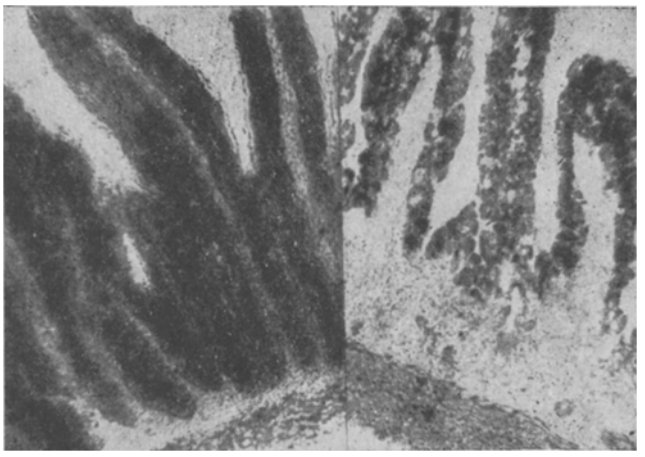

Fig. 2. Deposition of formazan in sections of the small intestine of control and experimental mice. Dose 4000 R, 72 h after irradiation. On the left) control, on the right) experiment. The remaining cells of the epithelium of the villi and the muscle layers are strained. The crypts are totally destroyed. 140×.

and fixation in formalin, were transferred to slides and mounted in glycerol-gelatin. The difference between the intensities of staining of the intestinal sections of the irradiated and unirradiated animals were determined by means of a comparator microscope and assessed by the signs +, ++, +++. The sign "0" denoted no difference.

EXPERIMENTAL RESULTS

It was found by the histochemical method used in the investigation that the wall of the small intestine is heterogeneous as regards succinate dehydrogenase activity. The crypts and the epithelium of the villi were characterized by high enzyme activity, weaker activity was found in the muscle layers, while the connective-tissue structures of the intestinal wall showed hardly any enzyme activity.

A characteristic localization of the enzyme was also observed at the cellular level. In the epithelial cells formazan granules were concentrated in greatest numbers in the apical portion, i.e., that facing the lumen of the intestine. In the basal part of the cells there were notably fewer formazan granules and the nucleus showed no enzyme activity.

The effect of various doses of radiation on the oxidation of succinate at various times after irradiation is illustrated in the table and in Figs. 1 and 2. The staining of the epithelium of the villi and crypts was taken into account. In column 1 of the table the intensity of staining of pairs of sections obtained from the intestine of control animals is compared.

It follows from column 1 of the table that in the overwhelming majority of cases the sections obtained from two intact animals of the same species in one experiment gave the same intensity of color. The differences (in one of the 8 experiments on rats and in 2 of the 9 experiments on mice) did not exceed one + sign.

Irradiation of mice in a dose of 600 R. With this dose no changes were observed in the formation of formazan granules in the sections from the intestine of the control and experimental animals sacrificed immediately after irradiation. Slight activation of the reaction of formazan formation was observed 1 h after the action of radiation, and after 2 h the activation effect reached its maximum for this dose. After 4 and 6 h the activation was less marked, and at the end of 24 h there was a tendency for the normal reaction to be restored.

Irradiation of mice in a dose of 2000 R. With this dose, as with the dose of 600 R, immediately after irradiation no effect of the radiation was observed on the activity of succinate oxidation. After 1 h the activating effect of the radiation was greater than at the same time after the dose of 600 R, and as with the dose of 600 R, the effect reached its maximum 2 h after irradiation, although it was much stronger.

In some experiments the increase in the intensity of staining of the sections from the irradiated animals by comparison with the control was assessed at +++ (see Fig. 1). The degree of activation of the reaction fell 24 h after irradiation, but it was still higher than the control level.

Irradiation of rats and mice in a dose of 4000 R. With this dose also, the effect of radiation on the oxidation of succinate was not apparent immediately after irradiation. Subsequently the character of the changes in the activity of the reactions differed from that with doses of 600 and 2000 R. Activation was not only present 1 h after irradiation, but it had reached its maximum (for this dose), and after 2 h the effect was somewhat weaker. Later, 48-72 h after irradiation, marked morphological changes began to appear in the mucous membrane of the intestine, so that it became difficult to assess the reaction. The crypts disappeared 72 h after irradiation. The epithelium of the villi also showed marked changes (Fig. 2). In the remaining areas of epithelium of the villi the activity of the reaction reached a high level. Another characteristic feature was that in the muscle layers of the intestine, the elements of which are most radioresistant and which no morphological injury could be detected by the method used, even 72 h after irradiation the formation of formazan during oxidation of succinate exceeded the control level.

It may thus be concluded from these results that whole-body x-ray irradiation of animals is accompanied by activation of the reduction of the tetrazolium salt during oxidation of succinate. A direct relationship was found between the rate of development of the activation effect and its degree, on the one hand, and the dose of irradiation on the other hand (600, 2000, 4000 R).

What is the mechanism of this effect? It appears that there are at least two possible explanations of the activating effect of radiation on formazan formation during oxidation of succinate. First, the effect may be due to activation of the succinate dehydrogenase, and second, it may be due to radiation injury to the electron transport system. This second explanation is based on the author's earlier experiments in which inhibition of the cytochrome system and of cytochrome oxidase was demonstrated following the action of ionizing radiation. Injury to the electron transport chain interrupts the flow of electrons to oxygen, thereby removing competition for electrons during the oxidation of succinate between oxygen and the tetrazolium salt, and facilitating its reduction.

The following observations support this hypothesis. Activation of reduction of the tetrazolium salt and inhibition of the cytochrome system are equally dependent on the dose and become stronger with time. In addition, the author has shown that inhibition of the cytochrome system caused by the action of NaCN, like the inhibition caused by irradiation, is accompanied by intensification of the reduction of nitro-BT during oxidation of succinate.

The results of this investigation are in agreement with other observations [11] according to which activation of the oxidation of succinate (as shown by the formation of formazan using another tetrazolium salt – INT) in the thymocytes of rats takes place during the action of radiation. The succinate-INT-reductase activity was intensified still further if the experiments were carried out in conditions blocking transfer of electrons to oxygen (in an atmosphere of nitrogen, the action of KCN). The results of the direct spectrophotofluorometric determination of the succinate dehydrogenase content as flavine showed that the enzyme concentration in the mitochondria of the thymocytes was unchanged after irradiation.

It may thus be concluded from the author's investigations and data in the literature that succinate dehydrogenase is a radioresistant system. The increase in the flow of electrons to the tetrazolium salt during oxidation of succinate in irradiated animals may evidently be regarded as the result of injury to the cytochrome system, which is sensitive to the action of ionizing radiation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
