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Inhibition of O_2 uptake of rat jejunum by X irradiation «in vitro». Protection by cysteamine *

by

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Among the effects of the ionizing radiations on man and the higher animals are various alterations in the digestive system. The intestinal absorption and various aspects of the metabolism of the small intestine may be affected. The corresponding investigations have been carried out by means of irradiation *in vivo* of the whole animal or of determined regions of the same.

Recently we have been able to prove that the irradiation of intestinal sacs kep *in vitro* in physiological solutions may determine a strong inhibition of the capacity of active transport of sugars (11). The irradiation doses for the purpose have to be particularly high (20,000 r) and the inhibition is of about 45 % in determinations made shortly after irradiating. This almost immediate action on the active transport may be related to alterations in the aerobe metabolism of the tissue, since the active transport is dependent on energy.

We have therefore studied in the present work the action of the ionizing radiations — X rays — on the O_2 uptake of strips of the small intestine of the rat. In other tissues effects of this type have been observed with high doses of X rays. In slices of rat liver 100 Kr are needed in order to produce a significant discrimination (2, 12). Slices of tumoral tissues are more sensitive to the radiations and the O_2 uptake diminishes with from 10 to 20 Kr (6, 12). Contrary to these results, in which the irradiation inhibits the O_2 uptake, an increase has been observed in slices of kidney with doses higher than 25 Kr (10). Other references corresponding to various biological materials have been collected by BACQ and ALEXANDER (3). Our experiments have revealed a decrease in the

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O_2 uptake of strips of the small intestine of the rat after irradiating with 10 to 20 Kr, but this does not appear if the irradiation is carried out in the presence of cysteamine.

Material and Methods

Male white Wistar rats of 200 to 280 g have been used. After 24 hours fasting, they were killed by being strick on the nape. After the abdomen was opened, a segment of proximal jejunum of about 15 cm was taken, carefully washed with medium, cut in its whole length and divided into a certain number of strips which were them suspended in Krebs-Ringer-Phosphate medium. One part of them was irradiated and the other one treated in the same conditions but without irradiating.

Irradiation was performed with an 50 kv Siemens Dermopan apparatus with a 1 mm Al filter, at about 1000 r.p.m. The liquid coat on the tissue was kept practically constant (about 1 mm).

All the strips were weighed and placed in Warburg flasks, one to each flask. The fresh weight of each strip was about 90-110 mg. The O_2 uptake was measured by the Warburg method (14), in atmosphere of O_2 and Krebs-Ringer-Phosphate medium. Determinations were made at periods of 60 min. until the respiratory activity ceased. O time was about 30 min. after the end of the irradiation.

In the experiments with cysteamine, this substance was present in the medium during the irradiation time. When the irradiation was finished, the strips were repeatedly washed in the same medium without cysteamine, before being introduced into the Warburg flasks, to remove all the residual cysteamine. The temperature of the medium during the irradiation was about 15° C.

Results

I. *Effects of X irradiation «in vitro» on the O_2 uptake of jejunum strips*

In each of the experiments the O_2 uptake of the irradiated strips was compared with other, non-irradiated, strips belonging to the same jejunal segment. With each dose of irradiation, experiments were carried out on groups of 5 to 10 animals, and a good coincidence of results was obtained, in spite of the fact that the strips coming from different animals showed absolute basal values of O_2 uptake which varied widley. The strips taken from the same animal on the other hand, gave closely coinciding values. For this reason, each of the figures shows comparative results obtained with strips coming from one animal only, corresponding to a specimen experiment.

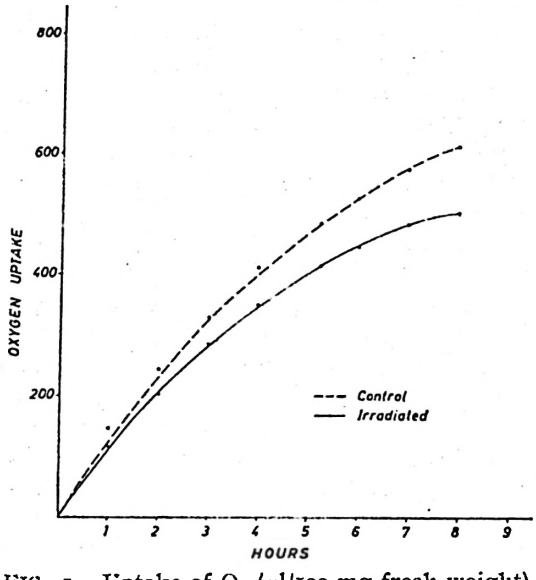

FIG. 1. Uptake of O_2 (μl/100 mg fresh weight) of strips of rat jejunum along the time in absence of external sustrate. Effect of 5,000 r X-iradiation.

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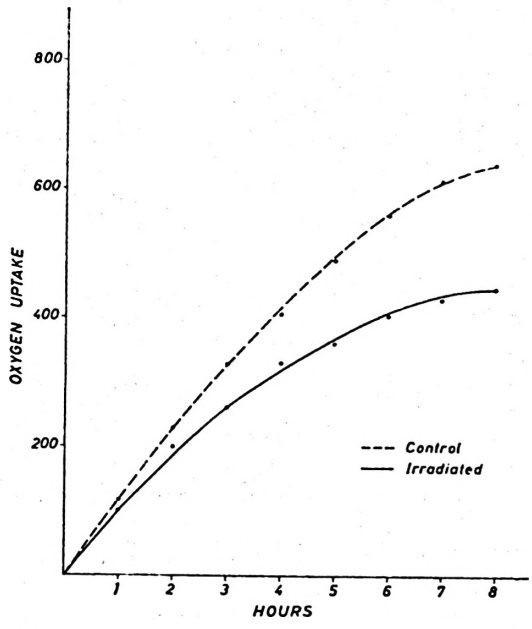

FIG. 2. Same experiment as Fig. 1 but with 10,000 r.

Each point is the mean value of the O_2 uptake of three strips from the same jejunal segment.

With 5,000 r (fig. 1), the irradiated strips breathe slightly less than the non-irradiated control strips. The difference becomes greater after an irradiation with 10,000 r (fig. 2), and is even much more appreciable after a dose of 20,000 r (figure 3). As may be seen in the figures, the slope of the curves becomes progressively less throughout the period in the irradiated strips than in the corresponding control strips.

It is evident that the irradiation of the strips provokes a decrease in the O_2 uptake which is hardly appreciable at the beginning of the experiment, but which appears more and more progressively and is very clear after about 2 or 3 hours of manometric recording.

Another group of experiments was carried out in similar fashion to the preceding ones, but with the difference that the medium in which the strips were immersed in the Warburg flasks contained glucose 2.77 mM. In these conditions the O_2 uptake increased through the presence of this metabolizable substratum (4).

The result of the irradiation with 20,000 r was very similar to that observed in the absence of substratum, with the difference that the slope of the curves of the control strips and of the irradiated ones was porportionally greater, on account of the aforesaid increase. That is, with levels of O_2 uptake notably greater than those of experiments without exterior substratum, the X irradiation of 20,000 r also produced a marked fall in the respiratory activity of the tissue (figure 4).

II. *Radioprotective action of cysteamine on the inhibition of O_2 uptake caused by X irradiation*

Some previous experiments revealed that cysteamine in 0.02 and 0.04 M concentration in the suspension medium

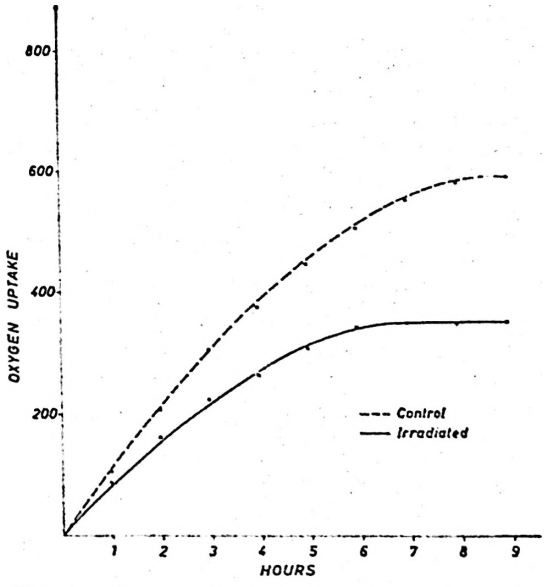

FIG. 3. Same experiment as Fig. 1 but with 20,000 r.

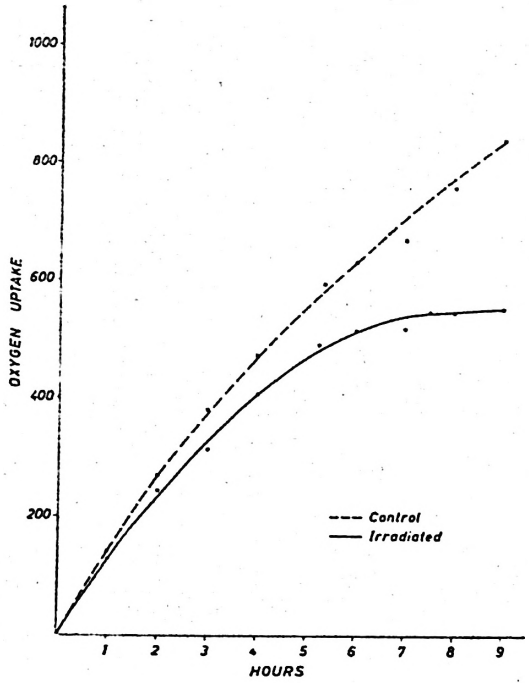

FIG. 4. Uptake of O_2 (μl/100 mg fresh weight) of strips of rat jejunum along the time, in presence of glucose 2.77 mM in the medium. Effect of 20,000 r X-irradiation.

of the strips produced a marked increase in the O_2 uptake, wich can be explained by its character as a substratum metabolizable by the tissue.

For this reason, in order to study the radioprotective action, the strips were suspended in a Krebs-Ringer-phosphate medium including cysteamine 0.02 M during the irradiation period and then, before passing the strips to the Warburg flasks, they were washed repeatedly in a medium which was identical but free of cysteamine, until the radioprotector was almost totally eliminated.

In one type of experiment we took from one and the same segment of intestine 3 strips as non-irradiated controls, another 3 which were irradiated in a medium with cysteamine, and another 3 which were not irradiated but which were subjected to a medium with cysteamine for the same length of time as the previous ones.

Figure 5 illustrates one of these experiments, in which it can be seen that neither the previous incubation with cysteamine nor the irradiation in the presence of cysteamine appreciably modify the O_2 uptake of the strips.

In other experiments 3 strips were used for control, another 3 were irradiated (20,000 r) in the presence of cysteamine and another 3 irradiated in the same way but without the radioprotector. Also in this case (fig. 6), it can be seen that the presence of cysteamine efficaciously protects the strips from the effects of the irradiation, at least as far as the O_2 uptake is concerned.

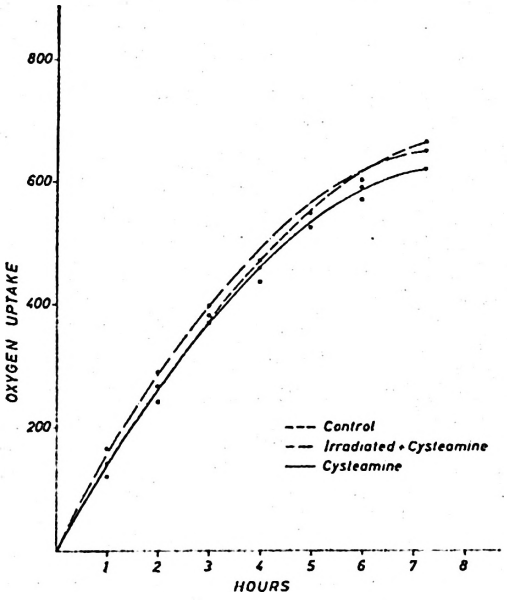

FIG. 5. Uptake of O_2 (μl/100 mg of fresh weight) of strips of rat jejunum along the time, in absence of external sustrate. Controls = not irradiated not incubated in cysteamine. Cysteamine = not irradiated but submitted to previous incubation with cysteamine 0.02 M. Irradiated + cysteamine = Irradiated (20,000 r) in presence of cysteamine 0.02 M.

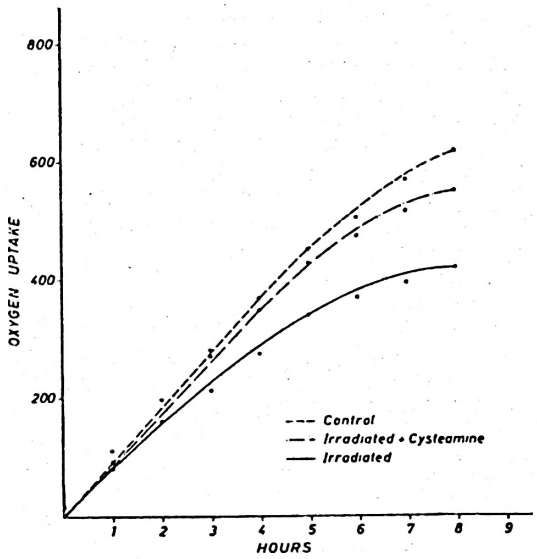

FIG. 6. Uptake of O_2 (μ/100 mg fresh weight) of strips of rat jejunum along time, in absence of external sustrate. Controls = not irradiated, not incubated in cysteamine. Irradiated = submitted to irradiation (20,000 r) Irradiated + cysteamine = irradiated (20,000 r) in presence of cysteamine.

Discussion

As we have seen, the O_2 uptake of strips previously irradiated *in vitro* diminishes in consequence of the irradiation. This inhibition appears with 5,000 r, becomes greater with 10,000 r and greater still with 20,000 r. These effects do not appear if the irradiation takes place in the presence of cysteamine 0.02 M in the medium, which thus shows a good radioprotective effect.

KIRRMANN and LE DOUARIN (8) had found that the irradiation *in vitro* of the embryonic intestine of the chicken with 7,500 r produced a decrease in O_2 uptake of about 20 %, an effect which did not appear if irradiation took place in the presence of cysteamine (7). However, according to their results the presence of cysteamine 0.05 M in the medium produces inhibition of the O_2 uptake (40 %), while in our strips of rat jejunum the O_2 uptake is increased when there is cysteamine in the medium, which has made it necessary to eliminate the cysteamine before determining the O_2 uptake.

Our experiments reveal that the inhibition of the O_2 uptake produced by irradiation becomes more marked with time. This may be due to the common observation that the biological effects of irradiation require a certain time before they appear and also that the lesion provoked leads to a reduction in the survival time of the tissue.

En 1951 MANOILOV (9) suggested that irradiation leads to a specific suppression of the aerobic phase of the respiration of the tissues due to damages in the enzymatic systems with Fe which participate in the respiratory chains. These ideas have been supported by later works (5), which, from different experimental points of view, seem to confirm that these biochemical lesions determine some of the effects of the ionizing radiations.

The same author (5) has shown, in the isolated heart of the frog, that the action of radiation specifically suppresses the function of these enzymes with Fe, perhaps through a disruption of the link between the heme and the proteins. In this connection, RICHMOND, ALTMAN and SOLOMON (13) have observed inhibition of the synthesis heme, and APPLEYARD (1), irradiating dry hemoglobin, deduced that there was a weakening or rupture of the link between heme and protein.

The inhibition of the O_2 uptake of the irradiated strips which has been observed in our results might be due, according to these ideas, to a lesion of the respiratory enzymes. In any case, whatever the biochemical lesion responsible for the respiratory inhibition may be, cysteamine appears once again as a very efficacious radioprotective substance,

Summary

A study has been made of the effect of X irradiation *in vitro* of strips of rat jejunum on the O_2 uptake of the tissue.

The X irradiation (50 KV, 1 mm Al, 1,000 rp.m.) produces a decrease in the O_2 uptake which first appears after 2 or 3 hours of recording and becomes more apparent as time goes on. The effect can already be noticed with 5,000 r and is greater at 10,000 and 20,000 r. We have also observed inhibition of the O_2 uptake when there is glucose (2.77 mM) in the medium.

The presence of cysteamine 0.02 M during the irradiation absolutely protects the preparations from these inhibitory effects on the O_2 uptake.

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