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Inhibition of the active transport of sugars by X irradiation «in vitro» of intestinal sacs *

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After the first works of WARREN and WHIPPLE (12), in wich it was conclusively shown that X irradiation coul cause death due to alterations of the intestinal epithelium, numerous studies have been made with a view to discovering the causes and mechanisms of the intestinal radioactive lesion.

Notable among the alterations of the digestive epithelium are those which are connected with a decrease in the velocity of cellular proliferation in the Lieberkhün crypts and, in consequence, in the velocity of «re-exchange» of the epithelial cells (3, 5, 10). Also norteworthy are the alterations in the motility of the gastrointestinal tract (4, 2).

The absorption upsets which may appear in the small intestine constitute one of the most important disturbances due to ionizing radiations (8).

It seems that no experiments have been carried out to study the effects of the irradiation of the intestinal sacs *in vitro*. In these working conditions a much higher dose of irradiation is usually necessary than *in vivo* in order that clear upsets may appear (1, 9). Nevertheless the irradiation *in vitro* excludes a great number of complex factors which may come into play when the live animal is irradiated.

In the present work we present the investigation carried out *in vitro* on the active transport of the small intestine of the rat using different doses of X irradiation, as also on the radioprotective effect of cysteamine.

Material and Methods

Wistar white rats were used, their weight varying from 100 to 160 gm, and kept fasting for 24 hours. Once killed, the proximal portion of the jejunum was isolated and a series of everted sacs was prepared according to the WILSON and

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WISEMAN method (13), the sacs being kept in a Krebs-Ringer-bicarbonate solution free of sugar and at a low temperature (0-6° C) until the experiment was begun. The fresh weight of these sacs was between 180 and 200 mg; 45 minutes after the animals were killed, and about 20 minutes after the sacs were prepared, part of the sacs were subjected to X irradiation with a 50 KV Dermopan apparatus fitted with a 1 mm. Al filter, producing about 1,000 r.p.m. During the irradiation the temperature of the incubating medium reached 11° C. The remaining sacs were kept in identical conditions except that they were not irradiated and were used as controls. 15 minutes after the irradiation had finished, 0.3 ml of Krebs-Ringer-bicarbonate solution, with galactose 5 mM, was placed in the serosal space of each sac and the sacs thus prepared were placed in Erlenmeyer flasks of a capacity of 50 ml, containing 5 ml of identical medium and in which carbogenous gas (95 % O_2 + + 5 % CO_2) bubbled at a constant flux (8 ± 0.2 1/hour). These preparations were placed in a thermoregulated bath at 38° C and were kept in these conditions of galactose in the serosal and mucosal for 60 minutes. The final concentration of galactose in the serosal and mucosal liquid was determined by the colorimetric method of Somogyi.

Results

Previous experiments showed that the active transport of galactose was well kept up for an hour if the sacs had been kept in a medium free of sugar and at a low temperature. If, on the other hand, they are kept at room temperature (18-20° C), the transport capacity decreases considerably in an hour.

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The capacity of active transport is expressed by means of the gradients of serosal (S) and mucosal (M) concentration which are obtained at the end of the experiment in accordance with the relation $\frac{S-M}{M}$, since in this way the expression of the inhibitions is more suitable than if the S/M quotient is established, and reflects better the fact that the increase in concentration produced in the serosal side is a consequence of the preferential passage of sugar against the gradient in the mucosal-serosal direction (7).

1. *Effect of X irradiation on the active transport of galactose*

X irradiation *in vitro* of jejunum sacs with 1,580 r does not reveal any effect on the active transport of galactose, the final gradients of concentration beign very much the same in the irradiated sacs and the control sacs. Nor is there any effect to be noticed when irradiating at doses of 3,000 r and 10,000 r. On the other hand, irradiating with 20,000 r we find a clear inhibition of the active transport of galactose This inhibition may be estimated as about 45 %.

Table I shows the results obtained.

TABLE I

Effect of X irradiation «in vitro» of everted sacs of rat jejunum on the active transport of galactose (5 mM initial).

Dose r	M	F	M-S/M	Diferences
Control	5.56	9.20	0.63	
1580	5.50	9.08	0.65	no sign.
Control	5.96	9.78	0.64	
3000	5.90	10.06	0.70	no sign.
Control	5.94	10.76	0.81	
10000	5.97	10.33	0.73	no sign.
Control	5.72	11.21	0.94	
20000	5.77	8.8	0.52	—44 %

For each of irradiation the mean value of the quotients $\frac{S-M}{M}$ was calculated for the irradiated sacs and for the control sacs which came from one and the same group of animals, and the changes observed were expressed in percentages of the activity of the respective control.

2. *Effect of cysteamine on the active transport of galactose*

Previously to the study of the possible radioprotective action of cysteamine, the sacs were incubated in a Krebs-Ringer-bicarbonate solution with cysteamine in the medium, and it was proved that the capacity of active transport of the sugar is not affected.

The incubation time was about 30 minutes, equivalent to that utilized in the irradiation experiments. The cysteamine was present in the medium at 0.1 M concentration. After incubation the sacs were washed repeatedly, the cysteamine being thus extracted. After undergoing this process, the sacs transported the galactose actively at the same intensity as the control sacs.

3. *Effect of X irradiation, in the presence of cysteamine, on the active transport of galactose*

Having seen the inhibition provoked by X irradiation (20,000 r) and proved that the incubation of the sacs in a medium with cysteamine did not modify their capacity of active transport of galactose, a study was made of the possible radioprotective action of the cysteamine.

For this purpose 8 sacs were prepared, the most contiguous possible, from the same portion of rat jejunum. Two of them, not irradiated, were used for control; two received 20,000 r; two were incubated in cysteamine but were not irradiated and the other two received 20,000 r, with cysteamine presente in the incubation medium. The sacs were placed in the medium with cysteamine 1 minute before beginnig the irradiation.

The results obtained with a good number of animals are grouped in table II. The cysteamine was used at 0.02 M concentration. As can be seen, the cysteamine in these conditions does not present a clear radioprotective effect. Other experiments, identical but with 0.1 M concentration of cysteamine, did not reveal radioprotection either.

Discussion

The results obtained permit us to affirm that the active transport of galactose from initial concentrations of 5 mM *in vitro* presented by everted sacs of rat jejunum previously irradiated, also *in*

TABLE II

Effet of X irradiation «in vitro» of everted sacs of rat jajunum on the active transport of galactose (5 mM initial). Influence of cysteamine 0.02

Dose r	M	F	M-S/M	Diferences %
Control	5.87	10.5	0.8	
No irrad. + Cist. 0.02 M	5.60	10.74	0.91	+14 (no sign.)
20000	5.85	8.35	0.42	—47 (sign.)
Cist. 0.02 + 20000	5.86	9.19	0.56	—30

vitro, is inhibited to approximate 45 % with irradiation doses of 20,000 r. These doses are much higher than those necessary to provoke strong upsests in the absorption when the whole animal, or only the abdomen, is irradiated *in vivo* (6), so that the two types of experiment cannot be related.

Our results, however, are interesting in that they reveal for the first time an immediate biochemical lesion provoked by the exclusive irradiation of the small intestinal sacs maintained *in vitro*, which hinders the process of the active transport of sugars.

The differences observed irradiating in the presence of cysteamine do not permit us to see any radioprotective effect in these working conditions. The lack of protective action of the cysteamine might be due in this case to the fact that a sufficient level of concentration is not reached in the tissue, as a consequence of the short incubation time of the sacs in the medium with cysteamine before the irradiation, as also to the low temperature at which this irradiation takes place, which undoubtedly hinders its penetration into the cells. Tests are now being done which may permit us to clear up this point.

Summary

X irradiation of everted sacs of rat jejunum maintained *in vitro*, at about 10° C, provokes an immediate inhibition of the capacity of active transport of galactose. For this very high doses (20 Kr) are required, and the inhibition is of about 45 %. With 1,580, 3,000 and 10,000 r, no effect has been seen.

The presence of cysteamine (0.02 M-0.1 M) in the medium during irradiation does not seem to exercise radioprotective action.

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