REVISTA ESPAÑOLA DE FISIOLOGIA
R. esp. Fisiol., 25, n.º 2, págs. 129-136. 1969

Department of Physiology and Biochemistry (C.S.I.C.)
Faculty of Sciences
University of Navarra
Pamplona (Spain)

Effects of the X-irradiation *in vitro* on the O_2 Uptake and on the Utilization of Glucose by the Intestine*

by

R. Jordana** and F. Ponz

(Received for publication on October 30, 1968)

A good deal of effects of the X-irradiation on the digestive tract have been reported and among them some alterations in the intestinal absorption of sugars and other metabolic disturbances have been observed (1, 8, 15). Most of the research in this field was carried out observing the effects produced by the irradiation of animals *in vivo*. According to NADAL and PONZ (13) the irradiation of *in vitro* intestinal sacs of rats (15.000-20.000 r) provoked an evident inhibition in the active transport of galactose.

Regarding to the metabolism of the carbohydrates, LANE et col. (9) reported a remarkable diminution of the respiratory quotient some time after the daily administration of 25 to 200 r. The basal uptake of oxygen in rodents changed very little by irradiation (12, 18). A moderate hyperglycaemia in fasting animals (23), a transitory increase in the hepatic glycogen (10, 20), and a moderate diminution of the elimination of carbon dioxide have been reported as effects of irradiation. Forty eight hours after the exposure to X-rays within the range of 1.000 r, the expired carbon dioxide diminished (16), the hepatic glycogen increased and the glucose and other sugars were oxidized to carbon dioxide in a lower proportion. Two of three days after the irradiation (600 r), the utilization of glucose by the intestine of rats was lower than usually (14). In brain slices of animals previously irradiated with 1.500 r the oxidation of the glucose to carbon dioxide and the production of lactate is also diminished, the synthesis of glycogen being remarkably increased (5). The analysis of these results becomes more difficult on account of the complexity of the factors that may interfere when the whole animal is exposed to

* This work has been supported in part, by The International Atomic Energy Agency Vienna (Contract No. 263/RB) and in part, by the «Ministerio de Educación y Ciencia».

** Fellowship of «Comisaría de Protección Escolar» (1966-1968).

irradiation; this is the reason why the research on the effects of radiation on tissues *in vitro* is particularly interesting.

Concerning to intestine, KIRRMAN and LE DOURAIN (7) reported a 20 % inhibition of the O_2 uptake after the irradiation (7.000 r) of the intestine of chicken embryo. BALASCH and PONZ (2) exposed strips of rat intestine to X-rays *in vitro* and observed that the oxygen consumption was falling for 5 to 8 hours after the exposure by the effect of doses of 10, 15 and 20 KR.

In the present paper * the effects of the exposure of *in vitro* strips of rat jejunum to X-irradiation on the O_2 uptake, the utilization of glucose and the production of lactate are reported. It was proved that the O_2 uptake, with and without external glucose, and the utilization of glucose diminished, meanwhile was not an evident variation in the production of lactate which obviously proves the impairment of the oxidative ability of the tissue.

Material and Methods

White rats, 140-200 g weight, of the Wistar strain were used for the experiments. They were killed with a blow on the neck after a period of fasting of 24 hours. A length of about 20-25 cm from the beginning of the jejunum was removed and carefully rinsed, inside and outside, with Krebs-Ringer-Tris solution. Several strips of jejunum of about 50-70 mg wet weigth were suspended in Krebs-Ringer-Tris medium.

The rinsing solution and the medium used was the Krebs-Ringer-solution, prepared as UMBREIT et col. described (21), but combined with a Tris-HCl buffer, adjusted for a pH 7.4 (11), instead of the phosphates buffer.

The irradiation was performed by a Siemens radiotherapy apparatus at 200 Kv, 15 mA, with an Al filter of 2 mm. The tissues were suspended in Krebs-Ringer-Tris medium, at constant temperature (37° C) and irradiated at a distance of 14 cm. from the focus. The dose of the irradiation was always of 20.000 r (1.000 r/min.).

The time elapsed between the weighing of the strips and the transfer of the irradiated strips into the Warburg flasks, including the irradiation time, was of 60 minutes (preincubation time in Krebs-Ringer-Tris medium).

The O_2 uptake was measured by the Warburg direct method (22), the carbon dioxide being fixed by a 10 % KOH solution. Before transfering the preparations to the Warburg flasks, they were carefully rinsed with Krebs-Ringer-Tris medium several times, in order to eliminate all traces of metabolites that might be accumulated. There were 2.5 ml of medium free from glucose or with 2.77 mM glucose. The determination was performed in an O_2 atmosphere at 37° C, shaking at 100 oscillations minute and 3.5 cm amplitude. Readings were made at intervals of 60 minutes for 1 to 7 hours. The results were expressed in μM of O_2 per 100 mg of wet tissue. The dry weight, as determined in a good deal of strips, was of 17 % $\pm$ 0.5 % of the wet weight.

The determination of the glucose and the lactate was performed at the end of each experimental period; the medium and the tissue were transfered into a Potter homogenizer; then, proteins were precipitated from the homogenized material (4). The glucose was measured by a colorimetric method with glucose-oxidase (6), and the lactate by the BARKER and SUMMERSON method (3).

* The experimental results of this paper are a part of the Doctoral Thesis of R. JORDANA, at the Faculty of Sciences, University of Navarra, 1968.

Results

1. *Experiments without glucose in the medium.*

The O_2 uptake was measured for periods up to 5 hours, and the lactate for periods of 1, 3, and 5 hours.

As Table I and Fig. 1 show, the O_2 uptake of the control strips is very regular for the 5 hours of the experiment, although it slightly declines progressively. This diminution of the O_2 uptake was less evident when the experiment was carried out with recently prepared strips (19), what allows to be explained by the effects of the pre-incubation at 37° C for 60 minutes against an air atmosphere. On account of the same fact, the O_2 uptake in the first hour after pre-incubation was 3.68 ± 0.07 μM/100 mg wet weight, while in the recently prepared strips it reached up to values of 4.51 ± 0.11 μM, the difference being of statistical significance ($P < 0.001$). The last value coincides exactly with the ones obtained by SHERRAT (17) in similar conditions.

The irradiated strips always had O_2 uptake values lower than the controls; the difference was getting more and more evident in the course of time. From the third or fourth hour onward, the tissue actually stopped respiration.

The effect of irradiation on the O_2 uptake is quite evident when the values

TABLE I

Effects of the X-rays (20.000 r) on the O_2 uptake and the lactate produced by strips of rat jejunum.

The strips were irradiated during the preincubation period (60 minutes) in Krebs-Ringer-Tris medium. The O_2 uptake, and the production of lactate were measured after the exposure during the incubation period in Warburg flasks in Krebs-Ringer-Tris medium. $\bar{x}$ = mean values expressed in μM/100 mg wet weight; ϵ = standard error in the mean; No. = number of tests. Statistics according the Student method.

	Time (hours)				
	1	2	3	4	5
	O_2 uptake (μM/100 mg w.w.)				
Control $\bar{x}$	3.68	6.55	8.70	10.72	12.25
ϵ	0.07	0.14	0.21	0.36	0.45
No.	138	94	81	53	48
Irradiated $\bar{x}$	2.54	4.54	5.90	6.62	6.80
ϵ	0.12	0.27	0.46	0.67	0.53
No.	35	22	16	8	8
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Differences %	29.05	30.68	32.18	38.24	44.48
	Final lactate (μM/100 mg w.w.)				
Control $\bar{x}$	1.09		1.01		1.18
ϵ	0.04		0.05		0.04
No.	39		30		42
Irradiated $\bar{x}$	1.16		1.14		1.07
ϵ	0.14		0.14		0.15
No.	9		9		6
Significance	$P < 0.6$		$P < 0.4$		$P < 0.5$
Differences %	6.42		12.87		9.32

TABLE II

Effects of the X-rays (20.000 r) on the O_2 uptake, utilization of glucose and production of lactate by strips of rat jejunum.

Strips were exposed to radiation during the preincubation period (1 hour) in a Krebs-Ringer-Tris medium. The O_2 uptake, the utilization of glucose and the production of lactate were measured after the exposure, during the incubation period in Warburg flasks in Krebs-Ringer-Tris/glucose 2.77 mM medium. The mean values ($\bar{x}$) are expressed in μM/100 mg wet weight; ϵ = standard error in the mean; No. = number of tests.

	Time (hours)					
	1	2	3	4	5	7
			O_2 uptake (μM/100 mg w.w.)			
Control $\bar{x}$	4.52	8.26	10.56	12.01	14.64	17.24
ϵ	0.10	0.15	0.33	0.62	0.70	1.07
No.	106	106	89	41	38	9
Irradiated $\bar{x}$	3.48	5.48	6.58	7.28	7.97	8.95
ϵ	0.12	0.24	0.29	0.31	0.25	0.54
No.	58	44	36	26	21	9
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Differences %	23	33.65	37.68	39.38	45.56	48.08
			Glucose utilized (μM/100 mg w.w.)			
Control $\bar{x}$	3.07		5.86		5.58	6.38
ϵ	0.16		0.19		0.37	0.45
No.	33		18		24	18
Irradiated $\bar{x}$	2.25		3.83		3.75	3.88
ϵ	0.28		0.33		0.33	0.35
No.	7		9		13	12
Significance	$P < 0.02$		$P < 0.001$		$P < 0.001$	$P < 0.001$
Differences %	26.71		34.64		32.79	39.18
			Final lactate (μM/100 mg w.w.)			
Control $\bar{x}$	3.26		3.56		3.13	3.78
ϵ	0.41		0.17		0.36	0.29
No.	17		32		19	12
Irradiated $\bar{x}$	2.14		3.06		2.52	3.65
ϵ	0.21		0.30		0.17	0.31
No.	7		6		15	11
Significance	$P < 0.02$				$P < 0.1$	
Differences %	34.36		14.04		19.48	3.43

obtained for the correspondent periods of 60 minutes are compared one another (Table III). The inhibition that in the first hour reached the 29 %, attained a 88 % in the fifth hour.

The total amount of lactate after 1, 3 and 5 hours of incubation always reached the same levels; no different values were appreciated by the effects of time or irradiation.

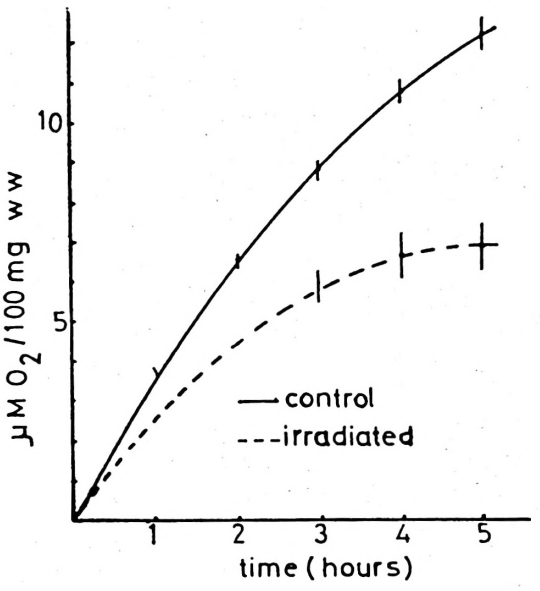

FIG. 1. Effect of *in vitro* X-irradiation (20.000 r) on the O_2 uptake by strips of rat jejunum. The vertical bars represent the standard error of the mean. Data from Table I.

2. *Experiments with glucose in the medium.*

These experiments were carried out using in the Warburg flasks the medium with 2.77 mM glucose. The O_2 uptake, the utilization of glucose and the total amount of lactate were measured at different intervals. The results are summarized in Table II and Fig. 2.

The O_2 uptake in the control strips was higher than in the experiments without glucose in the medium. The irradiation, as it happens in a medium free from glucose, produces a strong inhibition of the O_2 uptake. Comparing the values corresponding to the succesive intervals of 60 minutes, the inhibition was about a 23 % in the first hour and reached a 75 % in the fifth hour (Table III).

The glucose utilization in the control preparations was rather high, so that all the sugar available in the medium was nearly consumed in 3 hours. Irradiation clearly inhibited the utilization of glucose, the effect being already observed in the first hour. After three hours, the irradiated tissue is not more capable to metabolize the glucose that still is remaining in the medium.

The values of lactate found after several periods of incubation of the control strips, when glucose was present in the medium, were three times higher than in the expe-

TABLE III

Effect on the *in vitro* X-irradiation (20.000 r) on the O_2 uptake by strips of rat jejunum.

The strips were exposed to radiation during a preincubation period (60 minutes) in Krebs-Ringer-Tris medium. The data of the posterior oxygen uptake in media with or without glucose, correspondent to the succesive intervals of 60 min. have been calculated from those reported in Tables I and II. Differences between control and irradiated strips, expressed as per cent of the control values.

	Substrate	Successive Intervals of 60 minutes					
		1	2	3	4	5	7
Control	—	3.68	2.87	2.15	2.02	1.53	
Irradiated	—	2.54	2.00	1.56	0.72	0.18	
Differences %	—	29.05	30.31	36.74	64.35	88.23	
Control	Glucose 2.77 mM	4.52	3.74	2.30	1.45	2.63	2.60
Irradiated	» » »	3.48	2.00	1.10	0.70	0.69	0.98
Differences %		23.00	46.52	52.17	51.72	73.76	62.30

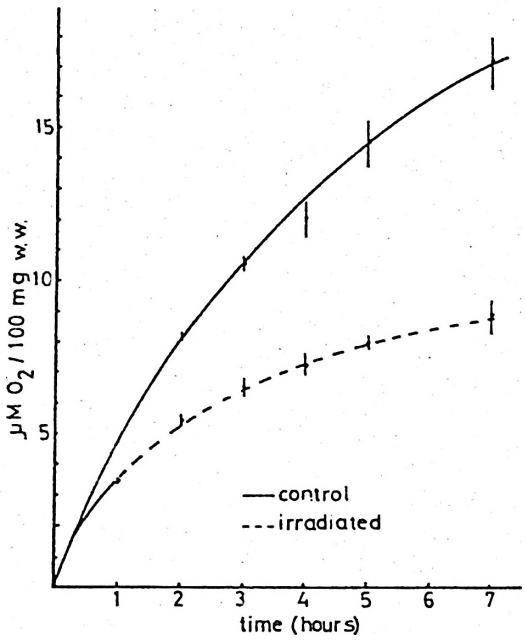

FIG. 2. Effect of *in vitro* X-irradiation (20.000 r) on the O_2 uptake by strips of rat jejunum incubated in Krebs-Ringer-Tris medium with 2.77 mM glucose. Vertical bars represent the standard error of the mean. Data from Table II.

riments without glucose. Irradiation did not affect the values of lactate, except in the results obtained after the first hour, when it seemed to be somewhat lower though the difference is of no good statistical significance.

Discussion

The previously refered (2) inhibitory effect of *in vitro* X-irradiation on the O_2 consumption of intestinal strips has been completely confirmed with much more experimental support. The inhibition of the O_2 uptake by irradiation is observed both with glucose in the exterior medium and without it. Therefore the action of the X-rays on the O_2 uptake is exerted independently from the inhibitory effect of the same dose of irradiation on the active transport of sugars (13). Irradiation must alter the respiratory systems by any mechanism and consequently diminishes the O_2 uptake by the tissue. This alteration is so important that a tissue exposed to radiation stops respiration in 3 or 4 hours.

The inhibition observed in the glucose utilization by a tissue previously exposed to radiation may be ascribed whether to a diminished capacity to oxidize the sugar or to the inhibition of the intake of glucose by the cell through its membrane. NADAL and PONZ (13), operating in similar conditions, demonstrated that doses of about 20.000 r *in vitro* inhibit the active transport of galactose up to a 45 %. The fact that the last mentioned inhibition is not complete and that the O_2 uptake is also inhibited at similar proportions in absence of external substrate, leads to accept that both factors take part in the diminution of the utilization of glucose.

It seems that irradiation inhibits the production of lactate for the first hour, although the statistical significance is not too much acceptable. However, the amounts of lactate in the irradiated strips as well as in the controls are very alike in periods of 3 or 4 hours. As the control strips consumend more glucose than the irradiated ones and as both groups of strips produced the same amount of lactate, it is to be inferred that there was a higher proportion of glucose transformed into lactate in the irradiated strips. In consequence of this assertion, it was observed that the lactate/glucose quotient increased with irradiation when the periods were longer than 1 hour. This alteration could be properly attributed to inhibition of the respiratory ability of the tissue, what facilitates a higher amount of glucose to be transformed into lactate because it cannot be used at the usual rate by oxidative ways.

Summary

The exposure of strips of rat jejunum mantained *in vitro* to X-rays (20.000 r), inhibits the posterior oxygen consumption of the tissues with or without glucose in the medium. The utilization of glucose also decreases, while the production of lactate is not noticeable modified. An explanation of these facts is given on the basis of the alteration of cell respiratory systems on account of the irradiation, independently of the inhibition produced on the sugar active transport.

References

1. BACQ, Z. M. and ALEXANDER, P.: «Fundamentals of Radiobiology». Pergamon Press, 1961.
2. BALASCH, J. and PONZ, F.: *R. esp. Fisiol.*, 22, 99, 1966.
3. BARKER, B. and SUMMERSON, W. H.: *J. Biol. Chem.*, 138, 535, 1941.
4. FRANCKEL, S. and REITMAN, S.: «Clinical Laboratory Methods and Diagnosis». C. V. Mosby Co., St. Louis, p. 99, 1963.
5. KAY, E. and CHAN, H.: *J. Neurochem*, 14, 401, 1967.
6. KESTON, A. S.: Abst. *Amer. Chem. Soc.*, 129, 31 C, 1956.
7. KIRRMANN, J. M. and LE DOUARIN, G.: *Comp. Rend. Acad. Sc. Paris*, 258, 2917, 1964.
8. KURTSIN, L. T.: «Effects of Ionizing Radiation on the Digestive System». Elsevier Publ. Amsterdam, 1963.
9. LANE, J. J., WILDING, J. L. RUST, J. M. TRUM, B. F. and SCHOOLAR, J. C.: *Radiat. Res.*, 2, 64, 1955.
10. LORAU, M. and LARTIGUE, O.: *Arch. Sc. Physiol.*, 4, 197, 1950.
11. MICHAELIS, I.: *J. Biol. Chem.*, 87, 33, 1930.
12. MOLE, R. M.: *Quart. J. Expt. Physiol.*, 38, 67, 1953.
13. NADAL, J. and PONZ, F.: *R. esp. Fisiol.*, 22, 105, 1966.
14. PERRIS, A. D., JERVIS, E. L. and SMYTH, D. H.: *Radiol. Res.*, 28, 1, 1966.
15. PONZ, F.: *Mem. R. Acad. C. y A. de Barcelona*, 37, 519, 1967.
16. RUST, J. H., LEROY, G. V., SPRATT, J. L., GAR BO HO and ROTH, LL.: *J. Radiat. Res.*, 20, 703, 1963.
17. SHERRATT, M. S. A.: *Comp. Biochem. Physiol.*, 24, 745, 1968.
18. SMITH, D. E., TYREE, E. B., PATT, H. M. and JACKSON, E.: *Proc. Soc. exptl. Biol. Med.*, 78, 774, 1951.
19. STAMPA, A. and PONZ, F.: *R. esp. Fisiol.*, 25, 69, 1969.
20. TOM, S. S.: Thesis, University of Chicago, 1956.
21. UMBREIT, W. W., BURRIS, R. M. and STAUFFEN, J. F.: «Manometric Techniques», Burgess Publ. Hing. C. Minneapolis, 1959.
22. WARBURG, O.: *Biochem. Zeitsch.*, 142, 317 152, 51, 1924.
23. ZUNZ, E. and LA BARRE, J.: *Comp. rend. Soc. Biol.*, 96, 125, 1927.