PHOSPHATASES IN TESTES AND EPIDIDYMIDES OF ALBINO RATS AFTER PARTIAL BODY γ -IRRADIATION

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(Received 23rd March 1971, revised 26th July 1971)

Our knowledge of the effects of ionizing radiation on the biochemical processes of testes and epididymides is meagre. Enzymatic studies of reproductive organs have been described (Ito, 1966; Gupta & Bawa, 1969), but the immediate effect of radiation in cell injury has been related to the curtailment of biosyntheses of nucleic acids and alterations in their chemical configuration. The events preceding the decrease of nucleic acid syntheses and other metabolic processes during irradiation are not understood. This communication describes the effects of localized γ -irradiation on phosphatases and their probable rôle in the regulatory mechanisms in the testes and epididymides.

Thirty, normal, white albino rats, weighing 140 to 200 g, were separated to form seven groups, consisting of four to five rats in each group. Rats were irradiated at a single surface dose of 2000 R from a cobalt-60 source shielded in a Theratron radiotherapy unit. The rats were anaesthetized by Nembutal and the scrotum was irradiated (70 to 80 R/min) at a distance of 65 cm from the source. Twenty-five sham irradiated animals acted as controls. The animals were decapitated at various intervals from 24 hr to 26 days after irradiation. The tissues from a group of animals were pooled, frozen, cut into pieces and randomized. About 1 g of the tissue was homogenized to give a 5 to 10% aqueous homogenate for enzyme assays. The method for acid phosphatase and inorganic phosphate have been described earlier (Gupta & Bawa, 1970). Alkaline β -glycerophosphatase was determined at pH 8.6 (veronal buffer); other conditions being similar to the acid phosphatase assay. Inorganic pyrophosphatase was determined by hydrolysing 0.042 M-sodium pyrophosphate by 0.1 ml enzyme at pH 8.4 (tris buffer, 0.1 M) using magnesium as activator for 15 min at 37°C. Adenosine triphosphatase was estimated according to Kielley (1954) at optimum pH 8.6 (tris buffer, 0.1 M). Analysis of 5'-nucleotidase was carried out at pH 9.5 using 1.0 M-glycine buffer (Heppel & Hilmoe, 1954) and glucose-6-phosphatase was assayed by the method of Swanson (1954) in citrate buffer at pH 6.2. The analyses of enzymes were performed in three to five replicates and the mean values after 7 and 15 days of irradiation for both the control and irradiated rats (five rats in each group) are reported in Table 1.

The activity of acid β -glycerophosphatase in the testes continuously decreased after 72-hr irradiation to values which were 82% of those of the control rats. On the 7th day after irradiation, a 32% decrease in the activity of acid β -glycerophosphatase was recorded. On the 15th day, this decrease was approxi-

TABLE 1
PHOSPHATASES IN THE TESTES AND EPIDIDYMIDES OF RATS AFTER LOCAL BODY IRRADIATION AT 2000 R

Exp. no.	Group	Time after exposure (days)	Tissue weight (g)	APase U(1)	Alk Pase U(1)	In PPase U(2)	G6Pase U(3)	ATPase U(2)	AMPase U(2)
Testes 1	R(5)	7	0.791	96.3 ± 2.5	86.3 ± 5.0	350.0 ± 30.0	51.0 ± 30.0	215.8 ± 11.0	573.0 ± 34.0 ^{N.S.}
	C(5)		1.180	143.3 ± 5.0	174.4 ± 4.0	1254.0 ± 10.0	65.6 ± 3.5	128.6 ± 10.0	530.0 ± 14.0
	R/C		0.67	0.68	0.50	0.28	0.78	1.63	1.08 ^{N.S.}
2	R(5)	15 to 16	0.586	47.5 ± 2.5	117.0 ± 4.0	604.0 ± 14.0	46.0 ± 2.0	149.2 ± 3.0	652.0 ± 24.0
	C(5)		1.241	152.5 ± 4.0	254.0 ± 12.0	1970.0 ± 15.0	63.5 ± 6.0	121.0 ± 5.0	466.0 ± 6.0
	R/C		0.47	0.31	0.46	0.30	0.72	1.24	1.39
Epididy- mides 1	R(5)	7	0.194	222.0 ± 6.0 ^{N.S.}	—	183.0 ± 15.0	31.5 ± 5.0	144.0 ± 11.0 ^{N.S.}	402.0 ± 13.0
	C(5)		0.222	221.0 ± 6.0	—	339.0 ± 20.0	44.4 ± 5.0	133.0 ± 5.0	311.0 ± 7.0
	R/C		0.87	1.00 ^{N.S.}	—	0.34	0.71	1.08 ^{N.S.}	1.29
2	R(5)	15 to 16	0.162	260.0 ± 5.0	—	199.0 ± 4.0	34.8 ± 8.3	200.3 ± 10.0	358.0 ± 12
	C(5)		0.242	367.0 ± 8.0	—	305.0 ± 5.0	52.4 ± 7.5	142.2 ± 13.2	280.0 ± 20
	R/C		0.67	0.71	—	0.65	0.66	1.40	1.28

U = Unit of enzyme activity is defined as μg of inorganic phosphate liberated by 100 mg of tissue in 1 hr (1), 15 min (2) and 30 min (3) at 37°C, respectively. N.S. = Values not significant. Other values were significant at 1 to 5% level.

R = radiated; C = control; APase = Acid β -glycerophosphatase; Alk Pase = alkaline β -glycerophosphatase; In PPase = Inorganic pyrophosphatase, G6Pase = Glucose-6-phosphatase; ATPase = Adenosine triphosphatase; AMPase = 5'-nucleotidase.

Figures in parentheses show the number of samples analysed.

mately 70%. In the epididymides, the loss in the activity of this enzyme was not appreciable in the early stages. Inorganic pyrophosphatase in the testes and epididymides seems to be more susceptible to radiation damage than acid β -glycerophosphatase and its activity after 24 hr was 80% in the testes and 85% in the epididymides. After 7 days, its activity in the testes was 32% and in the epididymides was 54% that of the controls. Correspondingly, the level of inorganic pyrophosphate in the testes was ten times higher than in the normal animals. Similar but less pronounced effects were observed in the activity of alkaline phosphatase and glucose-6-phosphatase. Published reports suggest that inorganic pyrophosphatase and alkaline phosphatase represent the same protein with different sites of actions (Fleisch & Russel, 1970). Specificities of microsomal glucose-6-phosphatase with inorganic pyrophosphatase of rat liver and kidney have also been described (Fleisch & Russel, 1970). Similar overlapping of various phosphatases seem to occur in the testes and epididymides. Changes in several phosphatases and inorganic pyrophosphatase in the epidermis of guinea-pigs has been demonstrated after β -irradiation (Tabachnik, Perlish, Chang & Freed, 1967). The rôle of inorganic pyrophosphatase in the regulatory mechanisms of biosynthetic processes has recently been reviewed (Fleisch & Russel, 1970). It is highly probable that during cellular death after ionizing irradiation, synthetic activities may be reduced due to the accumulation of pyrophosphates. During this process, the reversal of reactions for nucleic acid biosyntheses may be most sensitive (Gupta & Bawa, 1969, 1970). We believe that the loss of inorganic pyrophosphatase may be one of the initial events in cell injury due to ionizing radiation. The function of glucose-6-phosphatase in carbohydrate metabolism is known and it is possible that the loss of this enzyme might be one of the factors responsible for the accumulation of glycogen in the tests (Gupta & Bawa, unpublished observation). In human congenital Type-1 glycogen storage disease, it has been observed that this enzyme is almost lacking in both the liver and the kidney (Illingworth & Cori, 1965). Besides the loss of the activities of several phosphatases, the present report suggests a low but consistent increase in the activity of 5'-nucleotidase and adenosine triphosphatase. The increase in adenosine triphosphatase and 5'-nucleotidase after irradiation has been described in several mammalian systems (Ord & Stocken, 1961). It is possible that the loss of nucleotide precursors due to the increased activity of nucleotidases may be an additional event during the loss of nucleic acid biosyntheses after ionizing radiation. It is difficult to assess precisely the function of these enzymes because of the death of the germinal cells on the one hand and multiplication of interstitial and Sertoli cells on the other (Nebel & Murphy, 1960; Ito, 1966; Kochar & Bateman, 1969). It may, however, be concluded that the regulatory processes of the testes and epididymides are disturbed during irradiation, owing to loss of hydrolytic activities. Similar but less pronounced results have been observed at a sublethal dose of 720 R (unpublished data).

The authors are grateful to Dr P. N. Chhuttani, Director, and Dr J. S. Sodhi, Associate Professor, of the Postgraduate Medical Institute, Chandigarh, for providing irradiation facilities.

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