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Effect of Dimethyl Sulfoxide on Plasma Enzyme Changes in X-Irradiated Rats. (32158)

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(Introduced by P. D. Altland)
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Dimethyl sulfoxide (DMSO) has been found to have a radioprotective effect in mice(1) and rats(2). It has also been shown that DMSO, although causing little or no change in certain serum enzyme levels in normal animals(2,3,4), greatly augments the sharp rise in these enzyme levels occurring in rats subjected to various stresses such as arduous exercise(2) or exposure to high altitude(3) or a cold environment(4).

A number of investigators(6-11) have noted changes in serum enzyme levels following X-irradiation, but reports concerning the nature, degree and significance of some of these changes, particularly of serum transaminases, have varied and appear in part conflicting (8,9). The purpose of the present study was to determine the effect of DMSO on the enzyme levels in rats subjected to X-irradiation. It was thought that DMSO, perhaps by augmenting the changes, might aid in characterizing the alterations attributable to X-irradiation and indicate possible mechanisms involved.

Materials and methods. Several series of young adult male Sprague-Dawley rats weighing 275-375 g were divided into a saline group and a group treated with purified DMSO, provided by the Crown Zellerbach Corp., Camas, Wash. The number in the saline group was larger to compensate for a much higher mortality rate following X-irradiation (2). Each rat in the control group received an intraperitoneal injection of 10 ml/kg body weight of 0.85% saline (NaCl), while each rat in the other group was injected intraperitoneally with an equal volume of a 50% aqueous solution of DMSO, 5.5 g/kg.

The rats of both groups were given 800 r of whole-body X-irradiation beginning 5 to 15 minutes after the intraperitoneal injection. Irradiations were carried out at 23°C with an X-ray unit operated at 300 kvp and 20 ma with added filtration of 2 mm Cu. The target skin distance was 96 cm and the average dose rate about 38 r/min. Four saline and 4 DMSO-treated rats were irradiated at one time in a wooden cage partitioned into 8 compartments placed under the beam.

The animals were housed in a room maintained at 23°C and were given Purina laboratory chow and water *ad libitum*. Groups of rats were bled and sacrificed at 6 hours and 1, 2, 3, 6, 9, and 12 days after irradiation. Generally, the groups consisted of 3 saline- and 3 DMSO-treated irradiated rats and one non-irradiated control rat from each treatment group.

A blood sample for plasma enzyme determinations was obtained from each rat by cardiac puncture under light ether anesthesia immediately before sacrifice, using

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TABLE I. Plasma Enzyme Changes in Controls and X-Irradiated Rats Pretreated with Saline (C) or with One Dose (D) or 2 Doses (2D) of Dimethyl Sulfoxide.

Groups	No. of rats	GOT	GPT	AkP	Ald	LDH	MDH
			A. Nonirradiated controls				
C6hr	5	77 ± 10	29 ± 3	5.7 ± 1.4	12.9 ± 4.8	1133 ± 327	295 ± 79
D6hr	7	187 ± 33*	56 ± 8*	5.4 ± 1.7	31.2 ± 6.2*	826 ± 255	204 ± 32
C1d	4	91 ± 10	31 ± 6	8.9 ± .7	11.2 ± 2.2	741 ± 233	172 ± 57
D1d	3	141 ± 41	38 ± 8	7.3 ± 1.6	21.1 ± 2.5*	1099 ± 58	279 ± 147
C2d	6	75 ± 13	23 ± 2	8.3 ± .9	6.5 ± 1.1	554 ± 109	142 ± 43
D2d	6	135 ± 24	36 ± 5	5.8 ± 1.0	13.1 ± 1.4*	812 ± 155	294 ± 138
C3-12d	15	79 ± 4	33 ± 2	9.0 ± .7	10.3 ± 1.1	559 ± 129	192 ± 37
D3-12d	13	81 ± 4	34 ± 3	8.5 ± .4	11.5 ± 1.0	625 ± 80	224 ± 30
			B. Rats given 800 R (X)				
CX6hr	9	116 ± 14†	30 ± 1	6.1 ± 1.1	12.4 ± 1.2	994 ± 380	315 ± 116
DX6hr	8	466 ±121*†	103 ±25*	4.5 ± .5	41.6 ±10.7*	1718 ± 215†	1350 ± 342*†
CX1d	10	103 ± 9	24 ± 2	7.3 ± 1	12.9 ± 2.3	798 ± 108	158 ± 32
DX1d	8	277 ± 40*†	43 ± 4*	5.8 ± .9	25.2 ± 3.6*	845 ± 129	246 ± 97
CX2d	6	90 ± 10	19 ± 1	1.8 ± .4†	4.7 ± 2.4	731 ± 73	571 ± 234
DX2d	6	109 ± 12	27 ± 6	4.6 ± .9*	7.4 ± 1.4†	773 ± 157	278 ± 82
CX3d	10	41 ± 3†	11 ± 2†	.9 ± .2†	5.5 ± .9†	551 ± 185	184 ± 58
DX3d	13	54 ± 4*†	15 ± 3†	.8 ± .8†	7.6 ± 1.0†	482 ± 64	139 ± 21†
2DX3d	7	125 ± 14*†	16 ± 1†	1.0 ± .2†	16.7 ± 2.1*†	763 ± 109*	231 ± 66
CX6d	8	40 ± 3†	17 ± 2†	1.2 ± .4†	7.0 ± 1.7	314 ± 100	80 ± 16*
DX6d	7	57 ± 3*†	25 ± 2*†	3.1 ± .5*†	5.6 ± .5†	320 ± 123	83 ± 18†
2DX6d	7	65 ± 8	27 ± 5	2.3 ± .5†	6.9 ± .8†	176 ± 25†	46 ± 5†
CX9d	5	45 ± 5†	24 ± 5	2.6 ± 1.2†	6.5 ± .5†	92 ± 10†	59 ± 10†
DX9d	9	54 ± 4†	25 ± 1†	4.5 ± .6†	7.0 ± .2†	209 ± 52*†	131 ± 42
CX12d	3	61 ± 5†	19 ± 4†	4.2 ± 1.3†	23.8 ±19.1	221 ± 134	153 ± 123
DX12d	8	62 ± 10	22 ± 2†	4.7 ± .7†	8.3 ± 1.0†	305 ± 60†	149 ± 21

Group symbols are indicated in text and caption with the numeral on the right indicating hours (hr) or days (d) after treatment. Mean values ± S.E.M. are given in units/ml except that aldolase (Ald) and malic dehydrogenase (MDH) are given in milliunits/ml.

* Significantly different by Student's t test from values on line above in rats given no or less DMSO ($P < .05$).

† Significantly different from corresponding nonirradiated controls ($P < .05$).

heparinized syringes and tubes. A Beckman DU spectrophotometer was used for colorimetric measurements of aldolase (Ald) and lactic (LDH) and malic (MDH) dehydrogenases, and a Coleman Junior spectrophotometer for all other measurements. The plasma concentrations of glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined at 505 mμ by the Sigma-Frankel colorimetric method, and alkaline phosphatase (AkP) was determined at 410 mμ and LDH at 340 mμ, using materials and procedures obtained from Sigma Chemical Co.(12). Aldolase was measured at 540 mμ and MDH at 366 mμ, using Biochemica Test Combinations and Procedures (1964) obtained from C. F. Boehringer and Soehne, GMBH, Mannheim, Germany.

In some instances, plasma samples were applied to cellulose acetate strips, and the isoenzymes of LDH were separated by electrophoresis and identified by the use of nitro blue tetrazolium salt, as previously described (2).

Hematologic studies were made on a number of cardiac blood samples, using routine laboratory methods and a Coulter Counter, Model B, for white blood count determinations. The tissues of some animals were fixed in a 10% aqueous solution of buffered formalin (pH 7.0), and routine paraffin sections were prepared and stained with hematoxylin and eosin.

Results. X-irradiation caused a significant early rise in GOT in saline-treated rats and a rise in GOT, LDH and MDH in DMSO-treated rats. The values rapidly declined after 6 hours. The activity of all the enzymes, including AkP and aldolase, fell to subnormal levels 3-12 days after X-irradia-

tion (Table I). Compared to saline, DMSO caused a transient sharp rise in GOT and GPT and aldolase in non-irradiated rats and a rise in GOT, GPT, aldolase and MDH in the irradiated rats. The peak value at 6 hours after DMSO was 2-3 times that of the saline group in the non-irradiated rats and 3-4 times in the irradiated rats. DMSO did not prevent the fall in enzyme values noted 3-12 days after X-irradiation. A second dose of DMSO given 48 hours after irradiation and 24 hours before sacrifice (group 2DX3d, Table I) significantly elevated aldolase, GOT, and LDH values above those in rats given a single dose (group DX3d). A second dose given on day 5, however, had little effect on the plasma enzyme levels (group 2DX6d, Table I).

Generally, as reported previously(2), electrophoresis of the serum or plasma of normal rats reveals 2 LDH isoenzyme bands, a conspicuous band 5 nearest the cathode and a less intense band 1 nearest the anode. In the rat, isoenzyme band 1 is the major isoenzyme in the heart, and isoenzyme band 5 is the major isoenzyme in the liver, muscle and erythrocyte. In the DMSO group sacrificed 6 hours after irradiation, electrophoresis revealed all 5 SLDH isoenzyme bands. In all other groups, only normal isoenzyme bands 1 and 5 were noted.

Hematologic studies revealed marked differences between individual rats given the same treatment. The mean hematocrit value in 20 nonirradiated rats was 39.9 $\pm$ .5. Two saline-treated rats killed 9 and 12 days after X-irradiation had hematocrit values of 8 and 5, respectively. Hematocrit values in the other rats killed 9 or 12 days after irradiation ranged between 26.0-39.5 in the DMSO group and 22.0-37.5 in the saline group.

The mean leukocytes/mm^3 in 20 non-irradiated controls was 12,145 $\pm$ 569. At 9 days after X-irradiation, the mean leukocyte count was 1,997 $\pm$ 705 in 5 rats given saline and 1,979 $\pm$ 682 in 5 given DMSO. At 12 days, the mean leukocytes/mm^3 in 4 rats given saline and 7 given DMSO were 633 $\pm$ 125 and 4,058 $\pm$ 1,762, respectively. On day 12, all 4 rats given saline had leukocyte counts below 1,000/mm^3, whereas 5 of 7 given DMSO had counts above 2,000/mm^3. This suggests beginning recovery and is consistent with our previous finding that many DMSO-treated rats began to gain weight at this period and survived a 30-day observation period, whereas saline-treated rats continued to fail after 800 r whole-body X-irradiation and rarely survived more than 12 days.

Histologic studies revealed irradiation damage in both saline- and DMSO-treated groups involving the spleen, lymph nodes, intestine, and other organs similar to those described in the literature(13). There were no marked differences between the two groups in the character and severity of the radiation damage.

Discussion. Our studies show clearly that exposure of rats to 800 r whole-body X-irradiation results in a transient elevation in plasma GOT followed by a prolonged fall in various plasma enzyme levels. Pretreatment with DMSO greatly augments the rise but has little influence on the subsequent fall. These findings suggest that X-irradiation causes a transient selective increase in cellular permeability(14) followed by a more prolonged decrease(10). Because of the much higher concentration of enzymes in tissues (15), only a slight change in cellular permeability may induce marked changes in the plasma.

At 6 hours after 800 r, pretreatment with DMSO induced in rat plasma (Table I) about a 2-4-fold increase in levels of all enzymes studied, except SAkP, and the appearance of all 5 LDH isoenzyme bands, suggesting a contribution from multiple organs(3). The abnormal LDH isoenzyme bands disappeared in 24 hours, and the enzyme levels returned close to normal in 48 hours, indicating that DMSO has only a transitory effect on plasma enzyme levels. In non-irradiated controls, DMSO only increased GOT, GPT and Ald plasma values, and the increases were relatively less than in the X-irradiated rats (Table I).

In earlier studies, a sharp rise in certain serum enzyme values was induced in rats by a 5-hour exposure to arduous exercise (3) or to a simulated high altitude(4) or cold environment(5). Pretreatment with

DMSO markedly accentuated the rise in exposed animals but had little or no effect in unexposed controls. The rise was attributed to a widespread increase in cellular permeability. The similar findings in this study support the view(13) that X-irradiation may similarly cause an initial widespread increase in cellular permeability.

A transient serum elevation of GOT, beginning several hours after X-irradiation, has been reported previously in rats(9) as well as in other species(7,11). The elevations were correlated in time with severe pathologic changes in the lymphoid tissue, intestinal crypts and bone marrow, and these tissues were considered to be the major source of the excess GOT in the serum(9). In this study, the pathologic changes were similar. However, the severity of the pathologic changes, as in our earlier studies(3-5), did not closely parallel the severity of the enzyme elevations. It seems likely, therefore, that the excess GOT was derived not only from necrotic cells, but also from cells with a selective increase in permeability but appearing normal in routinely stained paraffin sections.

The fall below normal of most plasma enzyme values 3-12 days after X-irradiation suggests a decrease in cellular permeability during this period(10). This conclusion is supported by our finding that the percentage increase in plasma enzyme levels induced in 24 hours by DMSO given on day 5 (lines 2DX6d/DX6d) was even less than in non-irradiated controls (lines Dld/Cld, Table I).

Changes in tissue enzyme activity do not seem to explain satisfactorily the enzyme changes in the blood. Ludewig and Chanutin (6) reported earlier a similar marked fall in plasma AkP in rats 3-8 days after X-irradiation (600 r), but the fall was not correlated with tissue enzyme changes. They found AkP activity unchanged in the liver and kidney during this period and increased in the thymus and to a small degree in the spleen. Braun *et al*(10) found a fall in serum GOT and GPT in rats 2-12 days after total body irradiation with 600 r and considered the possibility that this fall was due to decreased cellular permeability induced by irradiation. They found fluctuations in GOT and GPT activity which varied in time of onset after irradiation, intensity and duration in different organs and did not closely parallel the serum enzyme changes.

The radioprotective effect of DMSO appears unrelated to its effect on plasma enzyme levels. DMSO initially raises the plasma enzyme levels; other radioprotective compounds reduce the rise in enzyme levels in irradiated animals(11). Pretreatment with DMSO did not prevent or significantly lessen a later fall in enzyme levels even though it greatly increased survival. The radioprotective effect of DMSO must be attributed, therefore, to other undetermined mechanisms (2).

Summary. Rats were given intraperitoneally 10 ml/kg 0.85% saline or 5.5 g/kg 50% dimethyl sulfoxide (DMSO), a radioprotective compound, before exposure to 800 r whole-body X-irradiation. Values of plasma glutamic oxalacetic (GOT) and pyruvic (GPT) transaminases, aldolase, alkaline phosphatase, and lactic and malic dehydrogenases were determined. In non-irradiated controls, DMSO elevated plasma GOT and GPT values at 6 hours and aldolase at 6-48 hours. Values were normal 3-12 days after DMSO. In irradiated rats given saline or DMSO, plasma GOT was higher than in non-irradiated controls at 6 hours and then fell, along with the other enzymes, to subnormal values at 3-12 days. Levels of GOT, GPT and aldolase were higher 6-24 hours after irradiation in rats given DMSO than in those given saline. A second dose of DMSO caused a proportionally similar elevation when given 2 days after irradiation, but had relatively little effect on enzyme levels when given 5 days after irradiation. Pretreatment with DMSO did not prevent the fall in enzyme values noted 3-12 days after X-irradiation and had no marked effect on histologic changes. It is concluded that X-irradiation causes an initial widespread increase in cellular permeability followed by a decrease, and that plasma enzyme changes do not explain the radioprotective action of DMSO and are unreliable guides in evaluating the possible radioprotective effect of different com-

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Rabbit Thyroid Metabolism in Tissue and Organ Culture.* (32159)

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Mosier(1) recently reported the inability to detect labeled iodothyronine in the tissue or media of surviving rabbit thyroid tissue incubated in media containing iodide I-131. He was able to detect labeled iodothyronine in surviving *rat* thyroid tissue however, under the same *in vitro* conditions. Previous reports of the metabolism of C^{14} tyrosine in media with surviving rabbit and beef thyroid tissue and tritiated tyrosine in media with sheep thyroid slices have been conflicting(1-5).

The results of the present studies fail to support the concept that a species difference exists in the metabolism of iodide I-131 by thyroid tissue. In addition, we have been unable to detect incorporation of C^{14}-labeled phenylalanine or tyrosine into iodotyrosines or iodothyronines by thyroid tissue grown in organ culture in the presence or absence of TSH.

Methods. Adult New Zealand female rabbits were fed Purina Rabbit Chow and given tap water *ad lib* for at least one week prio to study. Total thyroidectomies were per formed following administration of veterinary Nembutal. The thyroid tissue was placed i sterile Dulbecco's phosphate buffered salin containing potassium penicillin G (100 units/ ml) and in streptomycin sulfate (100 μg/ml) The thyroid tissue was immediately dissecte free of surrounding connective tissue an thinly cut, using micro dissecting scissors The method of organ culture was that o Fell and Robinson(6) and Trowell(7) wit modifications as previously described(8) ex cept thermally inactivated rabbit serum wa employed in place of human serum. Dupli cate cultures were performed on each tissu studied. As a control for each tissue, methi mazole (70 μg/ml) was added to the cultur medium of one of the duplicate cultures t prevent active incorporation of iodine int protein-bound forms by the thyroid tissue.

Tissue-culture medium 199(9) containin 10% of thermally inactivated rabbit serun was employed at pH 7.4. Carrier-free I-13 was added to this culture medium to yiel

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