Radioprotective Effect of Topically Applied Dimethyl Sulfoxide on Mice

Dimethyl sulfoxide's (DMSO) protective properties against biological damage caused by ionizing radiation have been studied by a number of investigators[1–4]. These animal studies were mostly concerned with *internal* administration of the chemical (injection). Very low toxicity of DMSO has been reported by KLIGMAN[5]. This low toxicity, though disputed as to some late possible chronic damage to the eyes of experimental animals[6], as well as the high penetrating power through skin surfaces, suggested to us the possibility of a *topically* applicable radioprotective agent.

Methods. CFW male mice, 50–60 days old, were used throughout the experiments. The animals were housed individually in cage units of a 5 · 4 cubicle arrangement[7], with unlimited access to standard mouse food and water. Experimentation was started approximately 1 week after arrival of the mice from the supplier.

Radiation exposure was carried out with X-rays generated at 225 kVp, 20 mA, and filtered with 1.0 mm Al + 0.5 mm Cu at a target-to-mouse distance of 88.5 cm. The dose rate was 9.0–11.5 R/min. The total dosages delivered to the mice of the various experimental series ranged between 700 and 760 R. 3 main experimental directions were pursued: (1) DMSO or sham treatment (H_2O) before and after irradiation; (2) DMSO or sham treatment before irradiation; and (3) DMSO or sham treatment after irradiation.

For this purpose the mice in each cage unit were divided into 2 groups, 10 DMSO and 10 water-treated. For the treatment with DMSO the mice were removed temporarily from the cage and each mouse placed in a special Lucite tube for immobilization leaving the tail exposed. The major part of the tail was then immersed in anhydrous dimethyl sulfoxide (Matheson Scientific Co.) for various lengths of time (see Table). Exactly the same procedure was carried out with the control animals, except that the chemical was replaced with distilled water.

The mice were then returned to their cubicles and the entire cage unit placed under the X-ray machine. The cages were rotated during X-ray treatment to keep non-uniform exposure to a minimum. Dosimetry was performed with a Victoreen 25 R condenser R meter. Furthermore, since the DMSO and sham-treated animals were irradiated simultaneously, the reliability for comparing mortality rates was further increased.

Immersion times of the mouse tails prior to and/or after irradiation were varied, ranging from 1–10 min. 2 groups of 10 mice each received 1 min treatment daily before and after irradiation and 1 group of 10 animals only after irradiation. One single treatment of 1, 5 and 10 min duration was given to groups of 10 mice each prior to irradiation and to 1 group after irradiation (5 min treatment). The detailed treatment schedule and radiation dosages are stated in the Table.

The mice were observed daily for mortality over a period of 30 days, and in some instances beyond 60 days. Appropriate non-irradiated but DMSO- or H_2O-treated control groups were kept also.

Results. The control mice (total of 80) exposed to DMSO or water only, showed no mortality during the duration of the experiments, regardless as to frequency and length of time of tail immersion.

Animals irradiated with 715 R and treated daily for 1 min with DMSO or H_2O before and after irradiation gave a 30-day survival of 7 out of 10 (DMSO) and 2 out of 10 (H_2O) respectively. When radiated with 760 R, 4 out of 10 (DMSO) and 2 out of 10 (H_2O) survived after 30 days. After 60 days only 1 mouse was alive in the water-treated groups while the DMSO-treated number remained unchanged (7 and 4 mice).

Single treatment of 1 min before radiating (740 R) showed survival of 6 (DMSO) and 2 (H_2O) out of 10 mice each. 5 min single pre-irradiation (720 R) treatment gave a survival of 6 (DMSO) and 1 (H_2O) animals from each of 10 mice, and 10 min immersion showed 8 out of 8 (DMSO) and 4 out of 10 (H_2O) survivors exposed to 745 R. Treating the animals daily for 1 min after irradiation with 700 R resulted in the survival of 4 (DMSO) and 3 (H_2O) mice. 1 DMSO and 2 H_2O mice survived a 714 R

Experiment No.	Agent	Pre-irradiation immersion time (min)	Post-irradiation immersion time (min)	X-ray dose (R)	Mice per group	Survival 30 (60) days post-irradiation
1	H_2O	1 d	1 d	715	10	2 (1)
	DMSO	1 d	1 d	715	10	7 (7)
	H_2O	1 d	1 d	0	10	10 (10)
	DMSO	1 d	1 d	0	10	10 (10)
2	H_2O	1 d	1 d	760	10	2 (1)
	DMSO	1 d	1 d	760	10	4 (4)
	H_2O	1 d	1 d	0	10	10 (10)
	DMSO	1 d	1 d	0	10	10 (10)
3	H_2O	1 sp	–	740	10	2 (1)
	DMSO	1 sp	–	740	10	6 (6)
4	H_2O	5 sp	–	720	10	1 (1)
	DMSO	5 sp	–	720	10	6 (6)
5	H_2O	10 spx	–	745	10	4 (2)
	DMSO	10 spx	–	745	8	8 (8)
	H_2O	10 spx	–	0	10	10 (10)
	DMSO	10 spx	–	0	10	10 (10)
6	H_2O	10 sp	–	760	10	5 (4)
	DMSO	10 sp	–	760	9	8 (8)
	H_2O	10 sp	–	0	10	10 (10)
	DMSO	10 sp	–	0	8	8 (8)
7	H_2O	–	1 d	700	10	3 (2)
	DMSO	–	1 d	700	10	4 (3)
	H_2O	–	1 d	0	10	10 (10)
	DMSO	–	1 d	0	10	10 (10)
8	H_2O	–	5 sa	714	10	2 (2)
	DMSO	–	5 sa	714	10	1 (1)

Last treatment (H_2O or DMSO) given 10 min before start of irradiation except where marked 'x' (5 min before irradiation); d = daily treatment 10 days prior and/or 30 days after irradiation; sp = single treatment prior to irradiation; sa = single treatment after irradiation. Animals treated after irradiation received first treatment 5–10 min after radiation exposure.

1 M. J. ASHWOOD-SMITH, Int. J. Radiat. Biol. *3*, 41 (1961).
2 O. Vos and M. C. A. KAALEN, Int. J. Radiat. Biol. *5*, 621 (1962).
3 B. A. BRIDGES, Int. J. Radiat. Biol. *5*, 101 (1962).
4 C. VAN DER MEER, P. W. VALKENBURG, and M. REMMELTS, Int. J. Radiat. Biol. *6*, 151 (1963).
5 A. M. KLIGMAN, J. Am. Med. Ass. 193, 140 (1965); *193*, 151 (1965).
6 Federal Register, Title *21*, 1, Part 3, November 25, 1965.
7 W. S. Moos, J. B. FULLER, and J. PLAGGE, Proc. Anim. Care Panel *11*, 17 (1961).

radiation exposure when treated for 5 min with DMSO or H_2O after the irradiation.

Discussion. Two main effects become quite apparent from this study (Table): (1) DMSO applied topically prior to lethal amounts of X-rays offers considerable protection. (2) Application of the chemical after irradiation does not alter survival significantly from that of control mice. Furthermore, a single treatment with DMSO for 1 or 5 min before irradiation is as effective in providing protection as daily immersions.

Zusammenfassung. Versuche mit röntgenbestrahlten Mäusen ergaben, dass bei Dimethyl Sulfoxid (DMSO) die Mortalität bedeutend reduziert war. DMSO wurde nur äusserlich und nur kurzfristig vor der Bestrahlung auf die Mausschwänze aufgetragen.

W. S. Moos and S. E. Kim

University of Illinois, Department of Radiology, Chicago (Illinois 60680, USA), June 24, 1966.

Spontaneous Occurrence of Aneuploidy in the Male Germ Cells of *Naja tripidians*

The concept that the diploid karyotype is constant for a species, especially in the case of germ cells, is fast withering. The constancy in the diploid complement for the male and female garnatures is a fairly well established fact; but occasional departures from it have also been reported[1,2]. The present communication reports the occurrence of aneuploidy in the testicular material of *Naja tripidians*. Material, which was collected from the suburbs of Jagadhari (Punjab), was pre-treated in hypotonic sodium citrate and subsequently stained and squashed after Melander and Wingstrand[3].

Diploid chromosome complement in *N. tripidians* is represented by 38 chromosomes which are differentiated into 10 macro- and 28 microchromosomes. In addition to the cells with normal counts, a fairly large number of plates with different counts (photomicrographs 1–6) ranging from 36 to 41 have been observed (Table). This variation has been mainly observed with respect to the microchromosomes, the macro complement remaining constant. While a large number of aneuploid configurations are encountered, at the same time regular polyploids, such as octaploids and tetraploids, are rather rare.

1 K. R. Lewis and B. Jhon, Chromosoma *10*, 589 (1959).
2 G. P. Sharma, R. Prashad, and M. L. Gupta, Cellule *65*, 295 (1965).
3 Y. Melander and K. G. Wingstrand, Stain Technol. *38*, 217 (1953).

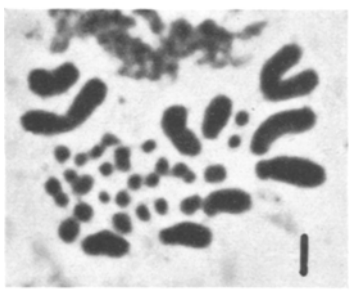

Fig. 1. Spermatogonial plate with 36 chromosomes.

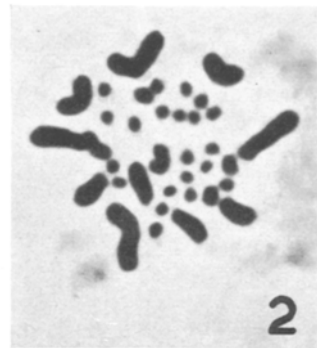

Fig. 2. Spermatogonial plate with 37 chromosomes.

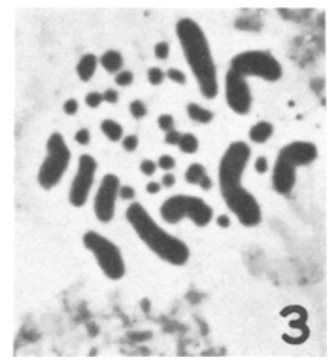

Fig. 3. Spermatogonial plate with 37 chromosomes.

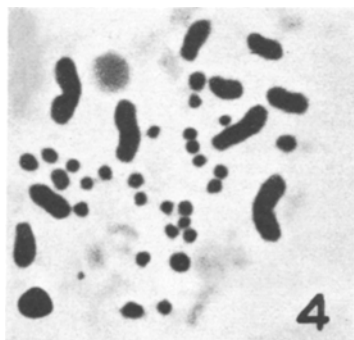

Fig. 4. Spermatogonial plate with 38 chromosomes.

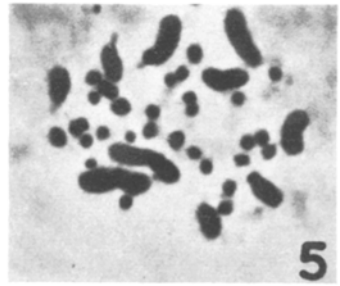

Fig. 5. Spermatogonial plate with 39 chromosomes.

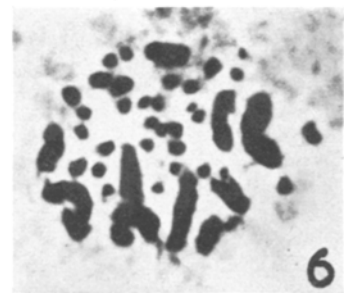

Fig. 6. Spermatogonial plate with 41 chromosomes. All. × 2000.