Protection by Serotonin-Creatinine Sulfate Complex of the Planaria *Dugesia tigrina* Against the Lethal Effects of X-Rays. (32534)

ALFREDO LAGUARDA-FIGUERAS AND RAFAEL VILLALOBOS-PIETRINI
(Introduced by S. G. Bradley)
Programa de Genética, Comisión Nacional de Energia Nuclear, México, 20, D. F. and Instituto de Biología and Instituto de Estudios Médicos y Biológicos, U.N.A.M., México 20, D. F.

The serotonin-creatinine sulfate complex (S-CS) has been used during recent years in numerous experiments as a radioprotective agent. The effectiveness of this substance is considered by Langendorff *et al*(1) as the best radiation protector known to date. For this reason, its mode of action has been studied extensively. Since the independent discoveries made by Bacq *et al*(2) and Gray *et al*(3) on the protection provided by S-CS, several hypotheses have been proposed to explain its mechanism. Many authors have postulated that S-CS produced tissue hypoxia as a result of vasoconstriction, and that it is the decrease in the oxygen tension in the tissues that causes the diminution of damage induced by radiation. This report presents data demonstrating that S-CS protects planaria from the lethal effects of X-rays. In these animals without a circulatory system, vasoconstriction cannot account for the observed radioprotection.

Material and methods. The planarians *Dugesia tigrina* used in the present experiments were collected from a natural pond on the grounds of the Botanical Garden (Universidad Nacional Autonoma de Mexico). In the laboratory, the planarians were maintained in dishes containing electro-purified water and were fed fresh liver twice a week. The experimental animals, 10 at a time, were placed for one hour in the S-CS solutions (Hycel, Houston, Texas); after the treatment they were returned to their regular dishes. The planarians were examined each day for 60 days and the dead planarians were scored and removed.

In the radioprotection experiments, the planarians were treated with 3.14×10^{-5} M S-CS (1:10 dilution of the stock) for one hour prior to X-irradiation. A Siemens Stabilipan was used as the source of X-irradiation; it operated at 250 KV and 15 mA, with a 0.5 Cu filter and an exposure rate of 119 roentgens per minute. Dosimetry was determined by a Siemens Universal Dosimeter. Different groups were irradiated with total exposures from 650 R to 2000 R. The control group was irradiated without any S-CS treatment. Each day for sixty days, we recorded the number of surviving and dead planarians.

Results and discussion. Eleven doses of S-CS (Table I) were tested and 5 replicates were made of each experiment; the curve for dose *vs* mortality was sigmoidal. It was found that 94% of all the deaths took place during the first day after S-CS treatment while the remaining 6% of deaths occurred between the second and fifth day after treat-

TABLE I. Survivals and Deaths of Planarians at Different Dilutions of the Stock Serotonin-Creatinine Sulfate Complex (S-CS).

S-CS (% of stock solution)	Molar solution ($\times 10^{-5}$)	No. of survivals	No. of deaths	Total	% Mortality
15	4.71	50	0	50	0
16	5.02	48	2	50	4
17	5.33	41	9	50	18
18	5.65	32	18	50	36
19	5.96	18	32	50	64
20	6.28	4	46	50	92
21	6.59	1	49	50	98
22	6.90	0	50	50	100
23	7.22	0	50	50	100
24	7.53	0	50	50	100

ment. Observations were made for a total of 60 days after treatment and no deaths were recorded after the fifth day. The mechanism by which the S-CS causes its lethal effects is unknown. In order to determine the dose that killed 50% of the planaria within 5 days ($LD_{50/5}$) we applied the Probit analysis(4). By this method an $LD_{50/5}$ of 18.2% of our stock solution was obtained.

As can be seen in Table II, the mortality

TABLE II. Lethal Effects Induced by X-Rays With and Without 3.14×10^{-5} M S-CS in *Dugesia tigrina*.

Exposure in roentgens	% Mortality without S-CS	Exposure in roentgens	% Mortality with S-CS
650	40	1200	20.0
700	40	1300	26.7
750	58	1400	46.7
800	62	1500	66.7
850	60	1600	80.0
900	100	1700	93.3
950	100	1800	96.7
		1900	96.7
		2000	100.0

increased from 40% to 100% as the X-ray doses increased from 650 R to 950 R. When the planarians were treated with S-CS and irradiated, the mortality increased from 20% to 100% as the X-ray doses increased from 1200 R to 2000 R. By Probit analysis (Fig. 1) we obtained the $LD_{50/60}$ in both cases. Without S-CS treatment, the $LD_{50/60}$ was 735 R and with S-CS treatment, it was 1384 R. The dose reduction factor (DRF) was

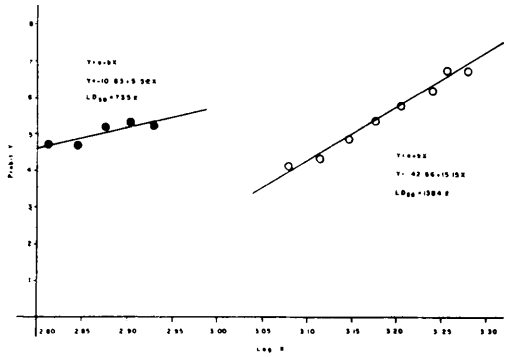

FIG. 1. Probit analysis of the influence of S-CS in the $LD_{50/60}$ to X-rays in *Dugesia tigrina*. X = dose, Y = % of Mortality. ● — ● X-irradiation; ○ — ○ S-CS plus X-rays.

1.883 which is somewhat higher than the DRF of 1.84 and 1.85 obtained by Langendorff *et al*(1) in mice.

The mechanism by which S-CS protects mammals has been attributed to its vasoconstrictive properties by Gray *et al*(3), Rothe *et al*(5), van den Brenk and Jamieson(6) and van den Brenk and Moore(7). In the planarian *Dugesia tigrina*, which has no circulatory system, vasoconstriction cannot account for the observed radioprotection. Other mechanisms proposed for the radioprotective action of S-CS are free radical scavenging(8), interference with biochemical reactions(9) and formation of a complex between S-CS and radiosensitive sites(10-12). It is not yet determined whether there is a single, universal mechanism of action of S-CS, or whether there is more than one mechanism operative. It will be necessary to examine each mechanism singly in order to determine its contribution to the radioprotective effect of S-CS.

Summary. The serotonin-creatinine sulfate complex (S-CS) at a concentration of 3.14×10^{-5} M protected the planarian *Dugesia tigrina* from the lethal action of X-rays; the dose reduction factor was 1.88. Vasoconstriction and the subsequent lowering of oxygen tension inside the tissues was not involved in this observed radioprotection.

The authors are indebted to Victor Tovar, Jesús Veláquez and Heriberto Gomez, Department of Physics, Unidad de Oncologia del Hospital General for the irradiations. The authors wish to thank Dr. S. Gaylen Bradley, University of Minnesota, for his helpful discussion and comments.

1. Langendorff, H., Melching, H. J., Ladner, H. A., Intern. J. Rad. Biol., 1959, vI, 24.

2. Bacq, Z. M., Herve, A., Bull. Acad. Roy. Med. Belg., 1952, v18, 13.

3. Gray, J. L., Tew, J. T., Jensen, H., Proc. Soc. Exp. Biol. & Med., 1952, v80, 604.

4. Finney, D. J., Probit Analysis, Cambridge Univ. Press, 1962.

5. Rothe, W. E., Grenan, M. M., Wilson, S. M., Nature, 1963, v198, 403.

6. van den Brenk, H. A. S., Jamieson, D., Intern. J. Rad. Biol., 1962, v4, 379.

7. van den Brenk, H. A. S., Moore, R., Nature, 1959, v183, 1530.

8. Alexander, P., Bacq, Z. M., Cousens, S. F.,

Fox, M., Herve, A., Lazar, J., Rad. Res., 1955, v2, 392.
9. Langendorff, H., Melching, H. J., Strahlenther., 1959, v110, 505.
10. Dukor, P., ibid., 1962, v117, 330.
11. Lohmann, W., Moss, A. J., Sanders, J. L., Porter, B. J., Woodall, D. M., Rad. Res., 1966, v29, 115.
12. Radda, G. K., Biochim. Biophys. Acta, 1966, v112, 448.

Received July 10, 1967. P.S.E.B.M., 1967, v126.

Stimulation of Rat Liver RNA Synthesis by Borate.* (32535)

ULRICH WESER† (Introduced by Harry G. Day)
Department of Chemistry, Indiana University,‡ Bloomington

The essentiality of boron in all plants that have been studied is widely recognized. Investigations regarding the mode of action of borate in plants indicate an involvement in nucleic acid metabolism. Thus RNA synthesis in beans and sunflowers appeared to increase when borate was present(1,2). Since it has not been established whether or not boron is essential in animals(3,4), it seemed to be of importance to study the effect of borate on RNA synthesis in rats. Liver RNA being rapidly synthesized can easily be labeled with [6-^{14}C] orotic acid, a precursor of RNA (5,6). This method allows the determination of even small changes in the rate of RNA synthesis. If borate should be involved in mammalian nucleic acid metabolism, this could be demonstrated by means of this labeling procedure.

Materials and methods. Three groups of male Sprague-Dawley rats, 5 per group with an average initial weight of 37 g, were restricted to a basal diet and environment extremely low in boron.

The basal diet was composed of: Hammarsten casein (Nutritional Biochemicals Corp.) 20%, reagent grade sucrose (Fisher Scientific Co.) 70%, salts 4%, Crisco oil 5%, vitamin mixtures 1%. The salt mixture and vitamin mixtures were essentially the same as used by Maurer and Day(7) in a study of the essentiality of fluorine in nutrition. The concentration of boron was not more than 0.001 ppm (10^{-7} M/kg), as determined using an adaptation of the procedure of Spicer and Strickland(8). The animals received highly deionized water. Groups 1 and 2 were kept on the boron-deficient diet, but Group 3 received the diet to which boric acid was added to provide 1 ppm boron (10^{-4} M/kg).

Six weeks later each animal from each group was injected intraperitoneally with 50 μC of [6-^{3}H]-thymidine (1.9 C/mM, Nuclear Chicago), and 9.5 hours later with 5 μC of [6-^{14}C]-orotic acid (44.5 mC/mM, Nuclear Chicago). However, the rats of Group 2 received simultaneously with each injection 20 μM borate as boric acid. After exactly 10.0 hours the rats were killed by decapitation and the well-bled livers were immediately removed and chilled in ice cold 0.32 M purified sucrose + 3 mM $MgCl_2$. The nuclei were isolated essentially as described by Higashi and Busch(5) and Widnell and Tata (9). The entire operation had to be performed within 24 hours. Aliquot parts (0.2 ml) of the nuclear suspensions were precipitated with 2% perchloric acid. The pellets were carefully washed with perchloric acid and ethanol and finally freeze-dried. To each pellet 0.5 ml hydroxide of Hyamine (obtained from Packard Instrument Co.) was added. After incubation for 6 hours at 37°, each suspension was diluted with 15 ml of a modified scintillation solution(10). Radioactivity was estimated in a liquid scintillation spectrometer (Packard Tri Carb model 3003) and was of the order of 10^{3} c.p.m. per sample. The liver nuclei suspensions of each rat were

* Supported by USPHS Grant AM 08209 03 to Harry G. Day

† Present address: Physiologisch Chemisches Institut der Universität, Tübingen, Germany.

‡ Publication no. 1523.