Failure of Zymosan to Increase Survival in X-Irradiated Mice.* (22286)

THOMAS J. HALEY, W. G. MCCORMICK, E. F. MCCULLOH, AND ANNA M. FLESHER.
Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California, Los Angeles.

Ross *et al.*(1) stated that the intravenous injection of zymosan in rats and mice was beneficial in radiation injury if given prior to irradiation, and detrimental if given afterwards. Although the effect obtained was related to the dose administered, the dosage range was not given. The mode of action of zymosan is related to its ability to combine with properdin, decreasing then increasing the serum content of this material. Properdin increases survival of irradiated animals by decreasing the post-irradiation bacteremia. Pillemer and Ross(2) gave information concerning the effects produced by doses of zymosan varying from 5 to 125 mg/kg. Finally Pillemer(3) reported that small intravenous doses of zymosan, administered to CF 1 mice, 24 hours pre- or post-irradiation, protected 70% of the animals against an $LD_{100/30}$ day dose of x-irradiation. These investigators did not give the characteristics of the irradiation used or the manner in which the mice were restrained during irradiation. Regardless of the paucity of information, the report of any therapeutic agent which is beneficial in radiation injury requires that further experiments be undertaken by other laboratories. We have conducted such experiments using both low and high doses of zymosan and are unable to substantiate the beneficial effects reported by Ross and Pillemer.

Methods. Six-hundred male, CF 1 mice, weighing an average of 24 g each, were arranged in groups of 20 animals each according to the design given in Table I. The Fleischman yeast zymosan solutions were prepared according to Pillemer and Ross(2). Two series of animals received intravenous injections of 5 and 125 mg/kg respectively, while another series received a 5 mg/kg injection intraperitoneally. Except during irradiation the animals were maintained in an air-conditioned room at 72 ± 5°F and were fed a diet of Rockland pellets supplemented weekly with additional vit. A and D. The 550 r radiation dose was administered from above and below the mice with two 250 KVP Picker Industrial Units operating simultaneously. The technical factors were: 250 KVP; 15 ma; FOD 100 cm; filters, 0.21 mm Cu inherent, 0.5 mm Cu parabolic and 1.0 min Al; HVL 2.02; size of field—total body; r/min measured in air 17.5 to 18.2. Both units were calibrated before and after each experiment with a Victoreen thimble r-meter. The animals were restrained in a plastic cage similar to the one described for guinea pigs (4). The results obtained were analyzed statistically by the Litchfield method(5).

Results. The results obtained with 5 mg/kg and 125 mg/kg of zymosan intravenously are given in Fig. 1 and Table I respectively. Intraperitoneal injection gave results similar to those in Fig. 1. The responses of the saline control groups in all series were similar to results obtained previously with other drugs(6-11). Post-irradiation medication with 5 mg/kg of zymosan either intraperitoneally or intravenously was highly detrimental to the mice, significantly reducing the ST_{50} day and day of total mortality in all cases. Pre-medication did not increase the ST_{50} day and the apparent 20% survival in the 7 day group is of doubtful significance because such effects have been observed in the control groups in past experiments. In fact, only 75% of the control animals of the intraperitoneal series died by the 26th post-irradiation day.

In the series given 125 mg/kg of zymosan, post-irradiation medication on days 1, 3 or 7 was highly detrimental decreasing the ST_{50}

* This article is based on work performed under Contract AT-04-1-GEN-12 between Atomic Energy Commission and University of California, Los Angeles.

TABLE I. Effect of 125 mg/kg of Zymosan Intravenously on Survival Time in Irradiated Mice.

Medication†	ST_{50}* and range in days	Slope and range	Final mortality %	Day
Saline control rad day	10.9 (9.45-12.55)	1.39 (1.26-1.54)	90	21
Z, 14 day pre-R	7.55 (6.56- 8.68)	1.39 (1.25-1.54)	95	14
Z, 7 "	10.4 (9.17-11.8)	1.34 (1.22-1.46)	90	21
Z, 3 "	9.65 (7.71-12.1)	1.60 (1.32-1.93)	75	13
Z, 1 hr "	11.8 (5.31-26.21)	1.57 (1.33-1.86)	75	20
Z, " post-R	12.2 (8.19-18.19)	2.31 (1.64-3.26)	65	20
Z, 1 day "	7.35 (6.39- 8.59)	1.38 (1.25-1.53)	100	18
Z, 3 "	5.55 (5.31- 5.85)	1.12 (1.08-1.16)	100	9
Z, 7 "	9.3 (8.65-10.0)	1.18 (1.12-1.25)	100	12
Z, NR control	—	—	0	21

* ST_{50} = Day on which 50% of animals alive. All values at P, 0.05; 20 animals per group.
† Z = Zymosan; rad day = radiation day; pre-R = pre-irradiation; post-R = post-irradiation; NR = non-irradiated.

day as much as 5.5 days over the control value. Pre-irradiation medication on days 14 or 3 also significantly reduced the ST_{50} day. Injections given 7 days or one hour pre-irradiation or one hour post-irradiation produced no significant increase or decrease in the ST_{50} day. However, total survivals in the latter 2 groups were 25 and 35% respectively as contrasted to only 10% survival in the saline control group. The statistical significance of these total survival figures is doubtful because of previous work as well as the 25% survival in the saline controls in the intraperitoneal series. This is also borne out by the wide ranges of both the ST_{50} days and the slope values.

Discussion. There can be little doubt that zymosan, in the doses and modes of administration reported herein, is not beneficial to irradiated mice. Smith(12) has also obtained similar results in mice. Thus, the work of Ross and Pillemer(1-3) cannot be confirmed. However, the conditions under which these investigators conducted their experiments have not been completely reported so that it is not possible to set up an exact duplicate of their work. On the other hand, the statistical design and overall range of conditions of the experiments reported herein should have been sufficient to uncover any beneficial effects which zymosan might have in radiation injury. Insofar as the mechanism of action of zymosan on the properdin system is concerned, the present work actually gives no insight into the problem because properdin titers were not measured. The increased rate of mortality observed with post-irradiation medication might have been the result of depletion of properdin in the blood. However, previous work from this laboratory(6-11) indicates that the irradiated animal reacts adversely to medication during the first post-irradiation week. Compounds like vit. E which are non-toxic in normal animals cause an increase in both the rate of and total mortality, if given after irradiation(7).

Summary. Pre-medication with 5 mg/kg of zymosan intravenously or intraperitoneally did not significantly increase survival in ir-

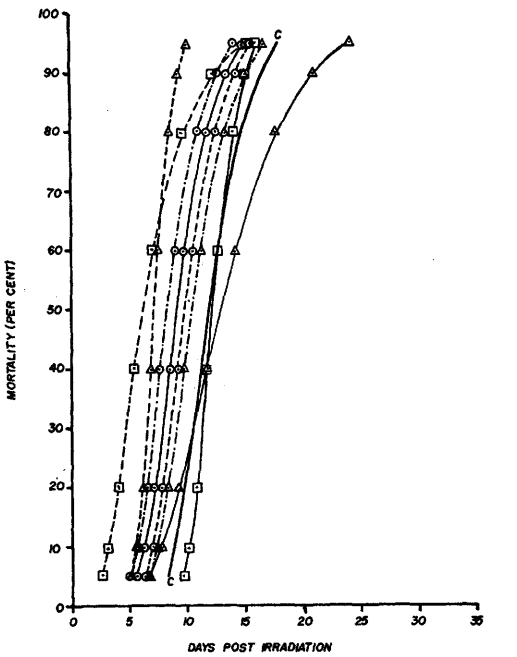

FIG. 1.

radiated mice. With intravenous injections of 125 mg/kg on pre-irradiation days 14 and 3, a significant reduction in the ST_{50} day was observed. Similar results were obtained with both doses when injections were given after irradiation. The beneficial effects of zymosan in the treatment of radiation injury in mice reported by Ross and Pillemer could not be confirmed.

1. Ross, O. A., Moritz, A. R., Walker, C. J., Wurz, L., Todd, E. W., and Pillemer, L., *Fed. Proc.*, 1955, v14, 418.

2. Pillemer, L., and Ross, O., *Science*, 1955, v121, 732.

3. Pillemer, L., *Trans. N. Y. Acad. Sci.*, 1955, v17, 526.

4. Haley, T. J., and Harris, D. H., *Science*, 1950, v111, 88.

5. Litchfield, J. T., Jr., *J. Pharmacol. Exp. Therap.*, 1949, v97, 399.

6. Haley, T. J., and Rhodes, B., *Science*, 1953, v117, 139.

7. Haley, T. J., McCulloh, E. F., and McCormick, W. G., *ibid.*, 1954, v119, 126.

8. Haley, T. J., McCulloh, E. F., and Heglin, J., *Radiation Research*, 1954, v1, 219.

9. Haley, T. J., McCulloh, E. F., and McCormick, W. G., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 195.

10. Haley, T. J., McCormick, W. G., and McCulloh, E. F., *ibid.*, 1955, v88, 475.

11. Haley, T. J., McCormick, W. G., McCulloh, E. F., and Heglin, J., *Radiation Research*, 1955, v3, 231.

12. Smith, W., National Institutes of Health, personal communication, October, 1955.

Received January 30, 1956. P.S.E.B.M., 1956, v91.

Effect of Hyaluronidase on the Cat's Brain. (22287)

J. L. ARTETA. (Introduced by C. N. H. Long.)

*Instituto de Endocrinologia Experimental, Madrid, Spain.**

The existence of interstitial spaces between elements constituting the nervous system has been assumed. Investigations of Bairati and Mattioli(1,2) confirmed and proved the existence of these interstitial spaces which probably were designed for the flow of cerebrospinal fluid. The experiments of Bairati(3), Bairati and Tripoli(4), Bairati and Patterno (5), Bartoli and Bertaccini(6), and Massari and Marsico(7) demonstrated by histochemical tests on different groups of animals the existence of a ground substance, the main element of which is a mucopolysaccharide or a mucoprotein contained in the interstitial spaces of the nervous tissue. The results of Hotchkiss' reaction were clearly positive in nervous tissue of fishes, amphibia, and lower mammals, whereas the results were doubtful when the test was performed on the brain of birds and higher mammals. These authors studied the behaviour of the nervous tissue under the influence of hyaluronidase and proved that the ground substance is affected by hyaluronidase, which allows a more rapid and extensive diffusion of the injected liquids and an easier mechanical dissociation of fragments of nervous tissue. Thus mucopolysaccharide may be present in interstitial spaces of the nervous substance. Freedman(8) also proved by means of histochemical reactions the existence of a substrate hydrolyzed by hyaluronidase, probably hyaluronic acid, in the nervous system, *i.e.* in the cerebral cortex, nucleus caudatus, and thalamus. Recently, Hess(9) stated too that the ground substance of the nervous tissue is a mucopolysaccharide, rather than a mucoprotein; since it is not hydrolyzed by pepsin, trypsin, or pancreatin. Hess found that hyaluronidase had no effect, and this indicates that this substance is not hyaluronic acid. In the present work, the behaviour of the nervous tissue under action of the hyaluronidase enzyme was examined.

Materials and methods. 13 young adult cats of both sexes were used, with a weight of 1.5-2 kg. All animals were operated on under

* Instituto Cajal, Madrid.