TABLE I. CD_{50}* of Two Penicillins in Hemolytic Streptococcus Infected Mice.

Penicillin used	1 dose therapy (mg)	2 dose therapy (mg)
Phenoxymethyl penicillin V	.207	.031
Penicillin G	.183	.030

CD_{50} computed by the method of Reed and Muench(1).

differing penicillin dosages in the range of 0.5 mg to 0.00095 mg. Stock solutions of the penicillins were furnished and prepared just before use in the mice by Dr. M. D. Bray of these laboratories. In one comparative test of the 2 penicillins, the mice got a single dose of penicillin, given at the time of infection. In the second test, a second dose of penicillin was given 5 hours after the first dose.

Results. As shown in Table I, a CD_{50} (dose curing 50% of mice) is indicated for the 2 penicillins from the results of these two tests. There was no significant difference in the therapeutic efficacy of phenoxymethyl penicillin V and penicillin G in the subcutaneous treatment of hemolytic streptococcus infections in white mice. The CD_{50} doses of the two were comparable, whether on single or double injection 5 hours apart. Furthermore the death rate in mice injected with subcurative doses of these two penicillins did not differ significantly.

Conclusion. Phenoxymethyl penicillin V and penicillin G have essentially the same curative properties against hemolytic streptococcus infections in white mice when these antibiotics are injected subcutaneously.

1. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

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Hemolysin Production in Irradiated Mice Given Spleen or Bone-Marrow Homogenate. (21978)

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Antibody formation is inhibited by exposure of the whole body to ionizing radiation (1,2), and the degree of inhibition is correlated with the time of antigen administration referred to the time of radiation exposure (3,4). Spleen or appendix shielding in rabbits during exposure to midlethal amounts of X-radiation resulted in a marked retention of the capacity to produce antibody to sheep erythrocytes(5). Preparatory treatment of the antigen with tissues or extracts of cells has recently been shown to improve the immune response in irradiated rabbits(6,7). Treatment of radiation injury in mice with spleen shielding(8) and spleen or bone marrow homogenates(9,10) accelerates hematopoietic recovery, lowers radiation mortality and has been shown to aid in the defenses against experimental infection(11). The present work was undertaken to study the hemolysin response in irradiated mice and to see whether the injection of marrow or spleen homogenate would hasten the recovery of antibody production following exposure to X-rays.

Methods. Mice, males of an inbred NIH strain, were 11 to 13 weeks of age at the time of irradiation. Mice of another strain, (BALB/cAn X $DBA_2J)F_1$, hereafter referred to as BALB/c, were used in one experiment. Littermates and treatment groups were distributed as nearly uniformly as possible within each group of 10 mice during the radiation exposure. Irradiation was carried out with a 200 KVP X-ray unit operating at 20 ma, with 0.25 mm Cu and 0.51 mm Al added filtration, HVL equal to 0.76 mm Cu. Radiation dose was 450 r (LD_4, 28 days) and in one experiment, 525 r, given at about 55 r per minute. Within an hour or 2 after irradiation, groups

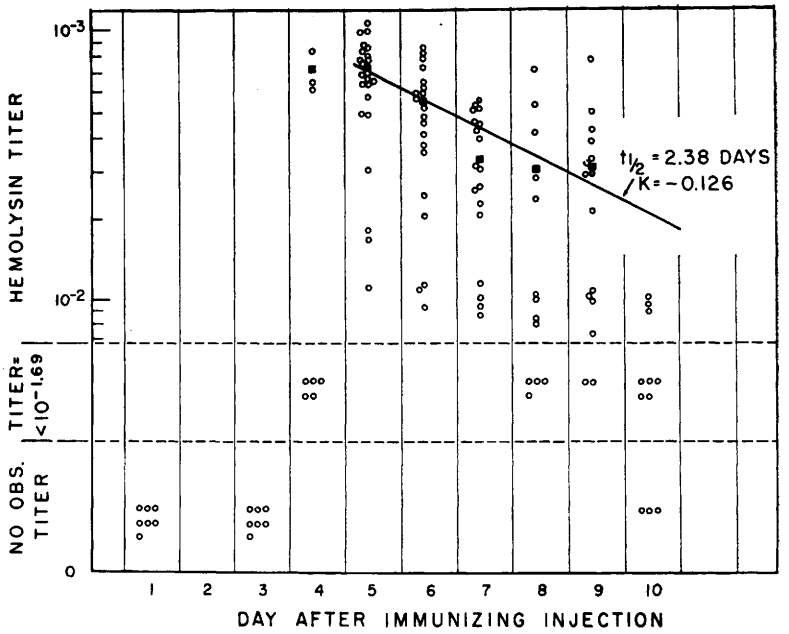

FIG. 1. Serum hemolysin titers of individual mice (○) sacrificed during the 10 day period following a single immunizing injection of sheep erythrocytes. Mean values for titers above 1:50 ($10^{-1.69}$) are denoted by (■), while titers below 1:50 or serums containing no observed hemolysin are shown schematically.

of the mice were given injections of suspensions of homologous spleen or bone-marrow from 4-week-old donor mice. Tissue, ground in a Potter type homogenizer and suspended in Tyrode's solution was injected intravenously. Each mouse received 0.5 ml of the suspension adjusted to contain the amount of tissue recoverable from the spleen or both femoral marrows of a single donor animal.

Mice were immunized with a single injection of a 2.5% suspension of washed sheep erythrocytes given intravenously at the rate of 0.01 ml per gram of body weight. This volume contained approximately 3.7 x 10^6 sheep erythrocytes which, from preliminary trials, was the lowest concentration that would yield satisfactory hemolysin titers in control mice. Serum from the individual mice collected at sacrifice was glycerinized and stored at about 3°C until the titrations were done within 3 to 4 weeks after the collection. Serum hemolysin content was estimated colorimetrically for individual mice following the method described by Taliaferro(12). Preliminary experiments had to be done to determine both the amount of the peak titer and the day upon which it would occur after a single immunizing injection. Experiments were then carried out to compare the peak serum hemolysin titers of irradiated homogenate-treated mice with their irradiated controls. In these experiments serum was collected on the 5th day after immunization from mice which had received their immunizing injection during the first or subsequent weeks through the 7th week after exposure to 450 r.

The possibility that the induction phase for the production of hemolysin might have been lengthened as a result of irradiation was tested in the irradiated homogenate-treated mice and their irradiated controls in other experiments. Serums of mice which had received their immunizing injection on the 4th, 5th, 6th or 7th week following exposure to 450 r were collected for hemolysin titration on the 5th or 6th through the 9th day after immunization.

Results. Initial phases of hemolysin production following a single injection of sheep erythrocytes are illustrated in Fig. 1, in which the titers are shown as the negative logarithms of the dilution per ml of the serum required

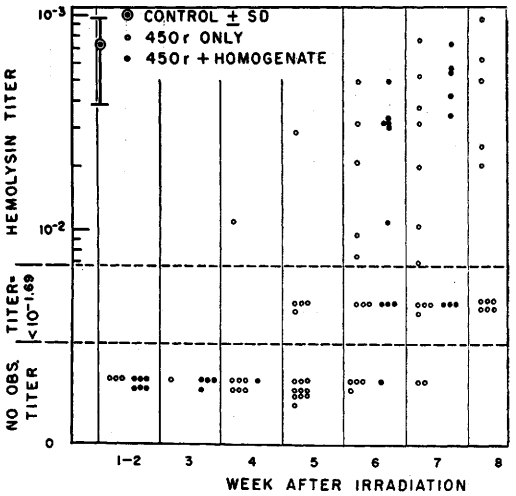

FIG. 2. Serum hemolysin titers on 5th day after immunization in marrow or spleen homogenate-treated irradiated mice compared with their irradiated controls. The mice were given a single immunizing injection of sheep erythrocytes during the indicated week following X-ray exposure and were sacrificed for the collection of their serums 5 days later.

to produce 50% hemolysis in a standard suspension of sheep erythrocytes. Serum samples in which the hemolysin content was just detectable, *i.e.* <1:50 or not detectable by our procedures, have been represented schematically in Fig. 1 and succeeding figures. The highest mean titer of the series, –2.73, corresponding to a dilution of 1:537 ± 333 (S.D.) per ml, occurred on the 5th day for serums of 23 mice. The slope of the line fitted by least squares through the means of titers above 1:50 for days 5 through 10 has the value –0.126. If it is assumed that group results reflect the changes in circulating hemolysin for any one animal, this slope represents a half life of about 2.4 days for the antibody.

Hemolysin titers on the 5th immunization day in spleen or marrow homogenate-treated mice are compared with their irradiated littermate controls in Fig. 2. Since no difference in hemolysin response was observed between the irradiated mice treated with bone marrow and those treated with spleen homogenate, the titrations of serums following these two treatments are given as for homogenate treatment. A gradual recovery of the immune response began during the 4th week after exposure to 450 r and continued at least into the 7th week (Fig. 2). The peak hemolysin titers in homogenate-treated mice agree closely with the titers of their irradiated controls and no indication of an accelerated return of the immune response is seen to be associated with the treatment.

Serums of irradiated or irradiated homogenate-treated mice showed little or no evidence of hemolysin production until the 4th week following exposure to 450 r. The homogenate treatment stimulated the early recovery of hematopoiesis since total leucocyte counts averaged significantly higher at 11 days after irradiation in 10 marrow and 10 spleen-treated mice than they did in 7 irradiated control mice

TABLE I. Hemolysin Production in BALB/c Mice Given Marrow or Spleen Homogenate after Exposure to 525 r.

	No. mice per group	Week following day of X-ray 1.5	3	4	5
Controls					
Hemolysin ± S.E.*	2–4		387 ±39	236 ± 50	287
WBC† ± S.E.	4	14.5 ±3.8		12.0± 1	
Spleen wt (mg) ± S.E.‡	4		175 ± 7.0	215 ± 8.5	253 ± 48.7
525 r only					
Hemolysin	9–10		—§	60	<50
WBC	10	.410± .05	1.8± .4‖	5.8± .6¶	
Spleen wt	9–10		223 ±82	124 ±101.6	124 ± 12.3
525 r + homogenate					
Hemolysin	15–18		—§	56	60
WBC	19	7.6 ± .5	7.9± .4‖	8.5± .6¶	
Spleen wt	17–19		130 ± 8.4	208 ± 23.4	188 ± 21.9

* Dilution/ml of mouse serum giving 50% hemolysis in a standard suspension of sheep erythrocytes. † Total leucocytes × 10^3/mm^3. ‡ Spleen wt in mg at sacrifice. § No observed serum hemolysin. ‖ WBC on day 18. ¶ WBC on day 26.

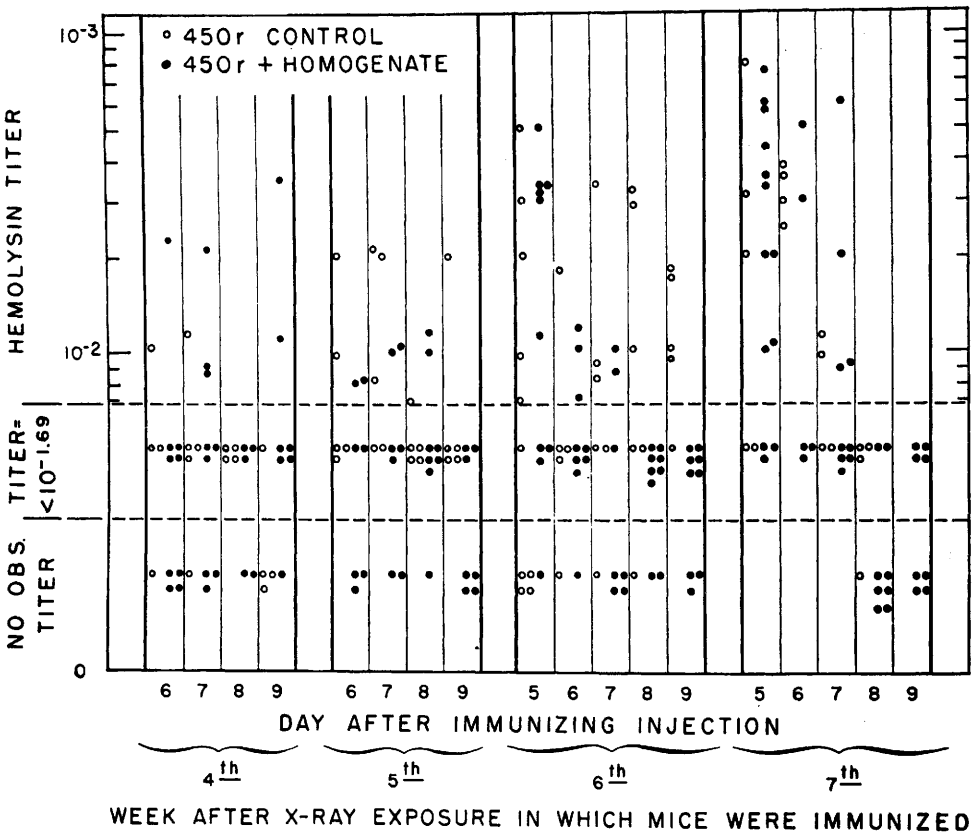

FIG. 3. Serum hemolysin titers in irradiated homogenate-treated mice and their irradiated controls for the indicated daily intervals during the 4th through 7th wk following exposure to 450 r.

(2145 ± 155, 2531 ± 591, 528 ± 110 cells per cu mm, respectively). Similar results were obtained in another experiment using BALB/c mice exposed to a somewhat higher radiation dose as shown in Table I. It is worthwhile to point out that the hyperplastic spleens seen in the third week (Table I) were not associated with improved hemolysin titers.

Fig. 3 summarizes the results of experiments which show that the time of maximum antibody production in the irradiated mice was not noticeably lengthened by the radiation exposure since there was no evidence that peak titers occurred later than the 5th day after immunization. The data of Fig. 3 indicate that the hemolysin titers tended to decline as the time from immunization increased, at least up to the 9th day. Furthermore, the homogenate treatment was without marked effect on the time of occurrence of maximum titer during the 4th through the 7th week after exposure to 450 r. The gradual recovery of the immune response following irradiation is shown in the higher individual titers and the relatively smaller proportion of low titered serums in the 7th week compared to the 4th and 5th weeks (Fig. 3).

Discussion. Homologous bone marrow and spleen cell homogenates, injected shortly after X-ray, fail to hasten the recovery of the production of anti-sheep erythrocyte antibody in irradiated mice. The favorable effect of the homogenate treatment on survival after exposure to X-ray doses in the lethal range is attributable in part at least to its salutary effect on the blood-forming tissues. The marrow and nearly every organ, as well as specific cell type, including lymphocytes and plasma cells, have been at various times considered the site of antibody formation (13). Although

homogenates of marrow or spleen either furnish cells which repopulate the hematopoietic tissue damaged by the radiation or supply a humoral substance which hastens the recovery of hematopoiesis(14), they produce no marked effect on the mechanism of hemolysin synthesis when given just after irradiation. Independent studies(15) of the cellular composition of the spleen and marrow of mice after exposure to 450 r have not shown unusual proportions of mature plasma cells either at 21 days when the spleens are enlarged or at 35 days when the antibody response has begun to recover.

More or less normal immune responses have recently been obtained in irradiated rabbits given antigen that had been incubated with lymph node cells(6) or minced spleen(7). In the experiments described in this paper (Fig. 2) antigen given as early as 2 days after the homogenate and within 4 days of irradiation did not result in a hemolysin response.

Summary and conclusions. 1. Recovery of the production of hemolysin in irradiated mice receiving bone marrow or spleen cell homogenate occurred at the same rate as in littermate control mice exposed to 450 r of X-rays. 2. Recovery of antibody production in response to injected sheep erythrocytes is gradual and begins in the 4th week after exposure of mice to 450 r. Recovery of the hemolysin response is not complete even at 7 weeks after this dose of radiation since many of the mice had peak serum hemolysin titers that were below the limits of non-irradiated controls. 3. The time required for the development of peak titer following a single immunizing injection was not lengthened by exposure to 450 r whole-body irradiation.

1. Hektoen, L., *J. Infect. Dis.,* 1915, v17, 415.

2. Taliaferro, W. H., and Taliaferro, L. G., *J. Immunol.,* 1951, v66, 181.

3. Hale, W. M., and Stoner, R. D., *Rad. Res.,* 1954, v1, 459.

4. Dixon, F. G., Talmage, D. W., and Maurer, P. W., *J. Immunol.,* 1952, v68, 693.

5. Jacobson, L. O., Robson, M. J., and Marks, E. K., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 145.

6. Harris, T. N., Harris, S., and Farber, M. B., *Fed. Proc.,* 1954, v13, 496.

7. Jaroslow, B. N., and Taliaferro, W. H., Abstract of paper presented at 3rd Ann. Meet. of Rad. Res. Society, May 1955.

8. Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J., and Eldredge, J. H., *J. Lab. and Clin. Med.,* 1951, v37, 683.

9. Smith, W. W., Marston, R. Q., Ruth, H. J., and Cornfield, J., *Am. J. Physiol.,* 1954, v178, 288.

10. Lorenz, E., Congdon, C., and Uphoff, D., *Radiol.,* 1952, v58, 863.

11. Marston, R. Q., Ruth, H. J., and Smith, W. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 289.

12. Taliaferro, W. H., and Taliaferro, L. G., *J. Infect. Dis.,* 1950, v87, 37.

13. The Nature and Significance of the Antibody Response, Symposium No. 5, N. Y. Acad. Sci., Columbia Univ. Press, 1953, 13.

14. Congdon, C. C., and Lorenz, E., *Am. J. Physiol.,* 1954, v176, 297.

15. Grenan, Marie M., unpublished observations from this laboratory.

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