Some Effects of Serum on Peripheral Leukocyte Count and Mouse Thymus After Exposure to X-Ray[1] (34307)

M. E. HODES, LILY C. YIP, AND PAO-LO YU
(Introduced by Donald E. Bowman)

Cancer Research, Departments of Medicine, Biochemistry, Psychiatry and Medical Genetics, Indiana University Medical Center, Indianapolis, Indiana 46202

During the course of studies on the prevention of leukemia by serum fractions it became obvious that some of these preparations could decrease or prevent the elevation of the specific activity of certain enzymes that follows whole-body irradiation (1). Prior to this Cividalli and Knyszynski (2) showed that spleen extract, which is able to prevent radiation leukemia (3, 4, 5), promoted regeneration of the thymus after X-irradiation. More recently, Berenblum *et al.* (6), demonstrated an earlier recovery of radiation-damaged bone marrow and thymus after injection of a fraction of serum containing α_2-macroglobulin. The correlation of these findings with the prevention of X-ray induced leukemia suggested further studies on the prevention or reversal by serum of the changes in blood and thymus that follow exposure to these rays.

Materials and Methods. The mice, X-ray exposure, and postirradiation care have been described before (5). Whole sheep serum, sheep serum albumin, or phosphate-buffered saline (PBS) were used for injection. All injections were given within 1 hr after exposure. Mice were exsanguinated under ether anesthesia into a heparinized syringe. The thymus was carefully dissected free of surrounding tissue, washed twice with 0.1 *M* phosphate buffer, pH 7.0, blotted dry between pieces of filter paper, weighed, and each 1 g was homogenized with 4 ml of buffer in a Teflon-in-glass homogenizer for 30–60 sec at 600 rpm. The extracts were centrifuged at 36,000*g* in the cold for 15 min. Enzyme assays were performed on the clear supernatants.

The DNA polymerase assay was performed according to Bollum (7) and RNA polymerase was determined by a slight modification of the method of Furth *et al.* (8). Total white blood counts (WBC) were performed on heparinized blood by the usual method. Protein was determined by the method of Lowry *et al.* (9). All determinations were performed at least in duplicate.

Results. The usual decreases in WBC, and in thymus weight and DNA polymerase activity were seen after single exposures to 300 R (Figs. 1–3) or 400 R (data not shown). Similar results were obtained in C57Bl/6 (Figs. 1-3) and Swiss (Table I) mice. Thymus RNA polymerase, by contrast was increased (Fig. 4). A single intraperitoneal injection of 0.25 ml of sheep serum caused a reversal, in whole or in part, of the radiation effects. The reversal is less marked in Swiss mice, and the difference in thymus weights between the serum and saline injected groups was not significant at the 5% level (Table I). The WBC response of Swiss mice was also delayed. Sheep serum albumin was generally without effect in either strain.

There are differences noted in the time of restoration to normal. Thus, whereas in each case the *overall* differences between serum and PBS or albumin treated animals were significant as tested in an analysis of variance, individual differences in enzyme activities are apparent at about the fourth day (Table 1 and Figs 1–4), in thymus weight at

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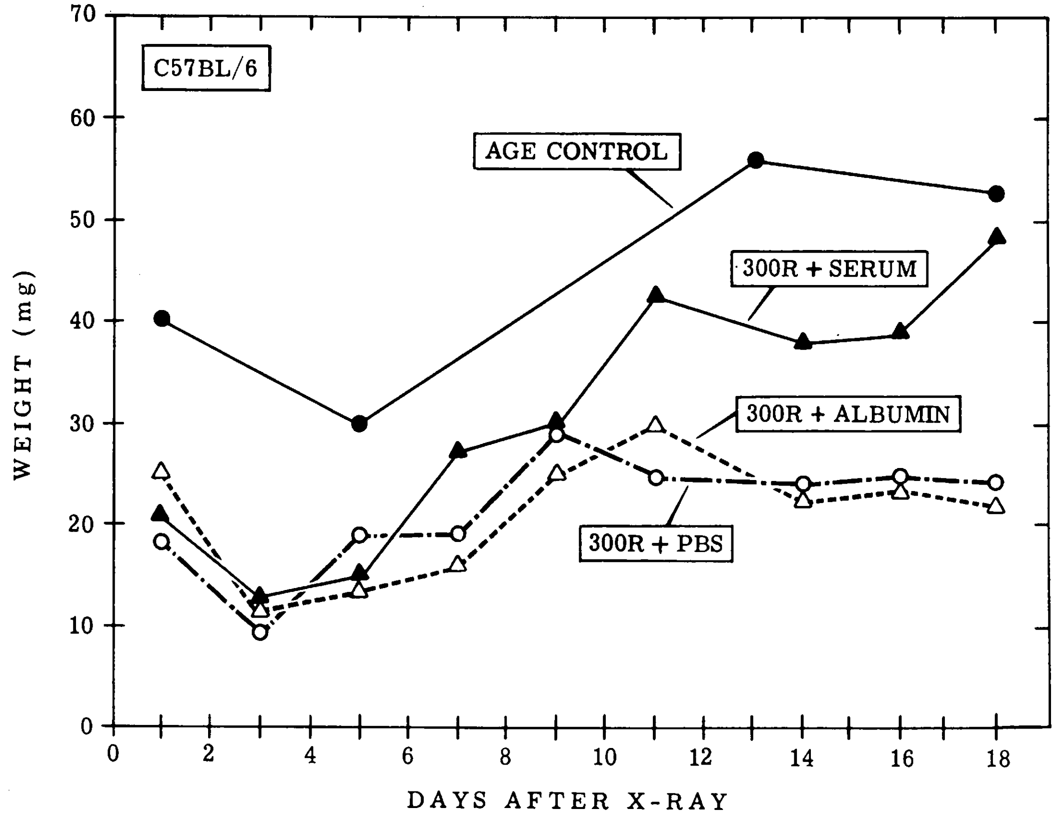

FIG. 1. The effect of serum or albumin on the changes in thymus weight after 300 R of whole-body X-irradiation of C57B1/6 mice; the age controls were not irradiated or injected.

about day 7, and in WBC not until the end of the second week. In repeat experiments the exact time is somewhat variable but falls within the ranges cited.

Discussion. The ability of serum and serum fractions to protect mice from otherwise lethal doses of irradiation, particularly when injected before exposure, was investigated by Stroud *et al.* (10). However, the reversal by postirradiation injection of serum or serum derivatives of the well known changes in enzyme specific activities evoked by irradiation seems to have been overlooked (1).

The relative decrease in specific activity of thymus RNA polymerase following injection of X-irradiated animals with serum correlates well with the decrease in lactate dehydrogenase (LDH) and alkaline phosphatase (AP) of liver, spleen, and serum under similar circumstances (1). In contrast to the elevation of RNA polymerase, DNA polymerase decreases after exposure, and is elevated upon subsequent injection of serum. This finding suggests an explanation for the increased incorporation of tritiated thymidine into cells, noted by Berenblum *et al.* (6), upon treatment with a serum derivative. The results with the thymus polymerases differ from our earlier findings (1), which revealed no significant changes in thymus LDH, AP, or DNase II.

Decreases in thymus weight following X-irradiation are well documented (10, 11). Restoration following injections of extract of sheep spleen was reported by Cividalli and Knyszinski (2). Kallman and Kohn (11) state that decreased food consumption could account for half this weight loss. This suggests that the injected serum might act by

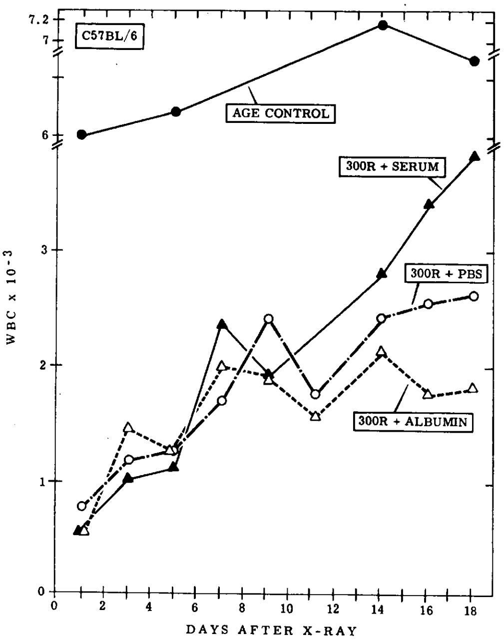

FIG. 2. The effect of serum or albumin on the changes in the peripheral total white blood cell count (cells/mm³) after 300 R of whole-body irradiation of C57B1/6 mice.

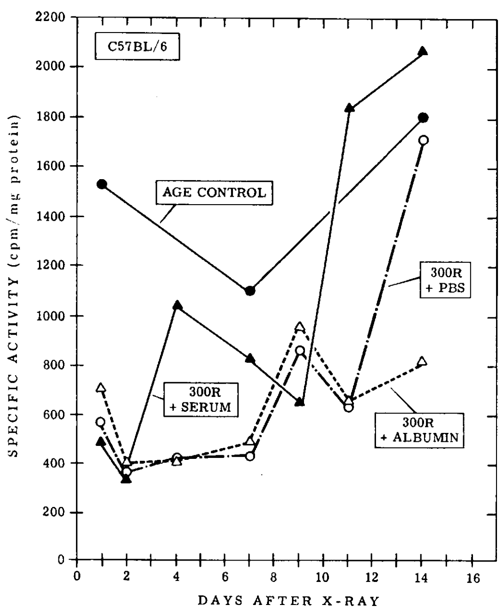

FIG. 3. The effect of serum or albumin on the changes in the specific activity of the DNA polymerase of thymus after 300 R of whole-body irradiation of C57B1/6 mice.

TABLE I. The Effect of an Injection of Serum or Albumin on Changes in WBC, Thymus Weight, and DNA and RNA Polymerase of Swiss Mice after 300 R.[a]

After X-ray (days)	Thymus Wt (mg) A	B	C	DNA polymerase (sp act) A	B	C	RNA polymerase (sp act) A	B	C	WBC (cells/mm³) A	B	C
1	41.1 ± 16.7	38.0 ± 10.3	37.3 ± 7.9	579.3	494.3	701.4	365.3	355.9	400.0	1280 ± 335	1320 ± 303	1600 ± 400
2	22.8 ± 4.9	22.8 ± 4.7	23.0 ± 3.7	364.8	346.5	396.0	320.7	289.2	363.3	1120 ± 481	1320 ± 481	1160 ± 260
4	25.6 ± 8.3	20.5 ± 2.7	27.1 ± 4.7	434.7	1040.2[ce]	742.8[d]	1423.9	491.2[ce]	1365.9	1080 ± 109	800 ± 141	1120 ± 179
7	33.2 ± 6.9	57.0 ± 9.3	37.8 ± 25.6	436.3	800.2[ce]	483.7	1192.1	817.3[ce]	1059.0	1320 ± 268	1880 ± 185	1800 ± 141
9	38.8 ± 19.1	42.2 ± 3.4	44.7 ± 15.4	869.4	606.1[e]	975.0	1092.5	420.4[ce]	891.2[d]	1600 ± 761	1640 ± 256	1840 ± 654
11	45.5 ± 20.3	58.6 ± 3.4	28.4 ± 15.9	682.8	1842.9[ce]	660.8	1521.5	1545.0[e]	869.3[d]	1880 ± 715	1680 ± 224	1800 ± 244
14	41.0 ± 13.5	61.7 ± 3.2	32.3 ± 18.7	1716.2	2073.5[ce]	825.9[d]	1384.9	2927.2[ce]	600.5[d]	2040 ± 172	3880 ± 300[ce]	2268 ± 587

[a] A = X-ray + PBS; B = X-ray + serum; and C = X-ray + albumin.
[b] Weights and WBC are stated as averages of 6 animals ± SD. The significance of the differences was tested in an analyses of variance. Differences significant at the 0.05 level are indicated thus: [c], A–B; [d], A–C; [e], B–C.

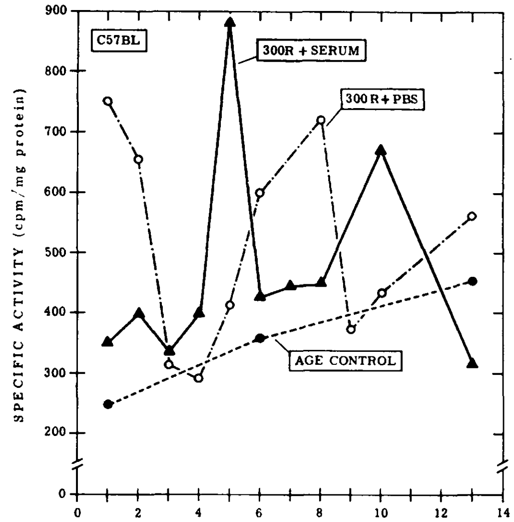

FIG. 4. The effect of serum on changes in the RNA polymerase of thymus after 300 R of whole-body irradiation of C57Bl/6 mice.

restoring the nutritional status of the mice. This is not a likely explanation, for serum albumin is obviously without effect. As noted, the weight loss is accompanied by a decrease in DNA polymerase but an increase in RNA polymerase.

The use of serum and serum fractions as radioprotective agents in mice was suggested by Stroud *et al.* (10). These preparations were much less effective if given after irradiation. Allen *et al.* (12) noted increased survival in rabbits given serum after irradiation. Survival of the animals after large doses of X-ray was the criterion chosen, and enzyme levels were not measured. [As suggested before (5) protection from radiation death and from the other effects of X-ray, such as leukemia, may depend on different mechanisms.]

Lorenz *et al.* (13) noted that the prolonged depression in WBC, particularly lymphocytes, after irradiation was not seen in mice whose spleens had been protected during exposure. Apparently effects similar to those obtained after spleen protection are elicited by injections of serum[2] (although only total WBC was measured in our experiments). Obviously serum must transport the humoral factor suggested by Lorenz *et al.* (13) as necessary for the rapid recovery of lymphoid tissues.

There is one important difference between the present experiments and those of Kallman and Kohn (11) and of Lorenz *et al.* (13). In the latter, a leukemia-inducing regimen of 150–225 R, given four times at weekly intervals, was used. As shown previously (1), responses in enzyme levels are evident after each exposure and injection. We therefore chose to use single exposures and injections in the present series of experiments. This perceptibly shortens the experimental period from 5 to 6 weeks or more to 4–5 days in the case of some enzymes, and to 2–3 weeks when the WBC is the test parameter.

The mechanism of action of the serum is not apparent. Serum does not prevent the secondary decrease in thymus weight recently stressed by Takada *et al.* (14). The relation of the changes observed to the leukemia-preventing ["RLP" (3)] activity of serum remains empirical. We have not tested the effects of substances other than serum and albumin on the various parameters reported here. Several individual components of serum or a mixture may be responsible for the effects noted here, and further work is under way to determine whether any of them possess RLP activity. The results to date suggest that one fraction is much more active than the remainder.

Summary. The C57Bl/6 and Swiss mice were exposed to 300–400 R of X-irradiation. The peripheral leukocyte count, thymus weight, and the specific activity of thymus DNA polymerase all decreased whereas the specific activity of thymus RNA polymerase increased above the levels of the age controls. A single injection of sheep serum, but not saline or sheep albumin, given within an hour after radiation hastened the return toward normal values. The differences in enzyme activities of the C57Bl/6 are apparent at about day 4, in thymus weight about day 7, and in leukocyte count at the end of the second week. The relation of these changes to

[2] We are grateful to Dr. D. Uphoff for suggesting measurement of changes in the WBC as an index of protection.

the prevention of X-ray induced leukemia is not yet apparent.

1. Yip, L. C., Blair, N., Yu, P. -L., and Hodes, M. E., Israel J. Med. Sci. **4**, 1188 (1968).

2. Cividalli, G. and Knyszynski, A., Radiation Res. **30**, 148 (1967).

3. Berenblum, I., Cividalli, G., Hodes, M. E., and Trainin, N., Nature **202**, 973 (1964).

4. Berenblum, I., Cividalli, G., Trainin, N., and Hodes, M. E., Blood **26**, 8 (1965).

5. Hodes, M. E., Clewell, D. B., Hubbard, J. D., and Yu, P. -L., Cancer Res. 26, 1780 (1966).

6. Berenblum, I., Burger, M., and Knyszynski, A., Nature **217**, 857 (1968).

7. Bollum, F. J., J. Biol. Chem. **234**, 2733 (1959).

8. Furth, J. J., Hurwitz, J., and Anders, M., J. Biol. Chem. **237**, 2611 (1962).

9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

10. Stroud, A. N., Brues, A. M., and Summers, M. M., J. Natl. Cancer Inst. **15**, 1109 (1955).

11. Kallman, R. F. and Kohn, H. I., Radiation Res. **2**, 280 (1955).

12. Allen, B. R., Wardell, H. G., and Clay, M., Science **123**, 1080 (1956).

13. Lorenz, E., Congdon, C. G., and Uphoff, D., J. Natl. Cancer Inst. **14**, 291 (1953).

14. Takada, A., Takada, Y., Huang, C. C., and Ambrus, J. L., J. Exptl. Med. **129**, 445 (1969).

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