2. Cochin, J., Axelrod, J., *J. Pharmacol. Exp. Therap.,* 1959, v125, 105.

3. Cochin, J., Economon, S., *Fed. Proc.,* 1959, v18, 377.

4. Sokoloff, L., Kaufman, S., *Science,* 1959, v129, 569.

5. Axelrod, J., *J. Pharmacol. Exp. Therap.,* 1956, v117, 322.

Received May 23, 1960. P.S.E.B.M., 1960, v104.

Effect of Pre-Immunized Rat Bone Marrow on Lethally Irradiated Mice. (25891)

PAUL H. CHIN AND MYRON S. SILVERMAN*

Biological and Medical Science Division, U. S. Naval Radiological Defense Lab., San Francisco, Calif.

Post-irradiation protection of lethally irradiated mice by injection of heterologous (rat) marrow is associated with rapid repopulation of the host's depleted hematopoietic tissue by the transfused marrow cells(1,2). The treated animals initially recover from radiation induced injury, but a significantly large percentage of deaths characteristically occur after third week of irradiation treatment(3,4). Although *direct immunological* evidence is still lacking, it is generally accepted that the pathogenesis of these "late deaths" is immunologic in origin. It is still uncertain whether the host or the transplanted graft initiate the immune reaction, nor is it known whether the pathological foundation of "late deaths" is circulating or fixed tissue antibodies. The experiments to be described were performed to compare time and incidence of deaths in lethally irradiated mice injected with bone marrow from normal rats and with bone marrow from rats immunized against tissues of the recipient strain of mice. On the basis of a graft *vs.* host reaction, it would be expected that both time and incidence of death would be accentuated following injection of pre-immunized rat marrow, resulting from direct restimulation of transplanted cells by the host's antigens. In contrast, if a host *vs.* graft reaction occurred or if colonization of immunologically active donor cells was unsuccessful no difference would result between groups receiving normal or pre-immunized marrow. Our results favor the latter conclusion.

Materials and methods. Sprague-Dawley albino rats, 12 to 18 weeks of age, were immunized against spleen and bone marrow suspensions prepared from normal adult LAF_1 ($(C57LxA)F_1$) mice. The mice were sacrificed by cervical dislocation. Spleens were excised, pooled, minced with fine scissors, then pressed through a 60x60 mesh stainless steel wire gauze. The cells were suspended and washed twice with chilled Tyrode's solution. Large clumps were removed by filtration through surgical gauze, and a cell count made in a hemacytometer. The desired concentration of cells was prepared by addition of Tyrode's solution. Mouse marrow cells were obtained by aspiration from the femurs and a suspension was prepared in Tyrode's solution. Three separate experiments were performed differing only in number of immunizing injections administered to the rat donors and in time after immunization at which the rats were sacrificed for collection of bone marrow and spleen cells. In the initial experiment, 2 intravenous injections of $1x10^8$ nucleated spleen cells were given 9 days apart followed by sacrifice 3 weeks after last injection. Serologic test of pooled sera from 10 rats showed a hemagglutinating titer of 1:512 against washed LAF_1 mouse rbc. In the second experiment, rats were given 2 intravenous injections of $1x10^8$ spleen cells a week apart followed by a third injection 2 weeks afterwards. These donor animals were sacrificed

* Opinions or assertions contained herein are those of authors and are not to be construed as official or reflecting views of Navy Department or of Naval Service at large.

TABLE I. Mortality and Mean Survival Time of LAF_1 Mice Receiving 870 r Whole Body X-Irradiation Followed by Injection of Immunized or Non-Immunized Rat Cell Preparations.

Exp.	Treatment of irradiated LAF_1 mice	No. of mice per group	% mortality (days) 30	60	90	120	150	Mean survival time of decedents (days) 30	60
21	1×10^8 imm. RBM*	25	56	80	80	80	80	18.9	26.5
	" non-imm. RBM	25	32	60	80	80	80	14.4	26.5
44	7×10^7 imm. RBM†	41	56	80	95	98	98	17.8	23.5
	" non-imm. RBM	39	46	74	74	80	87	21.7	27.2
48	" imm. RBM‡	35	29	69	80	86	89	14.9	27.6
	" non-imm. RBM	26	31	69	85	89	96	12.9	27.2
44 sp.	7×10^7 non-imm. RBM + 1×10^7 imm. rat spleen cells‡	18	44	72	72	78	78	20.2	27.9
	7×10^7 non-imm. RBM + 1×10^7 non-imm. rat spleen cells	28	36	64	71	75	75	19.3	27.8
48 sp.	7×10^7 non-imm. RBM + 5×10^7 imm. rat spleen cells‡	15	100					8.1	
	7×10^7 non-imm. RBM + 5×10^7 non-imm. rat spleen cells	15	100					12.0	

* Donor rats given 2 intrav. inj. of LAF_1 spleen cells.
† " " " 3 " " " " " " .
‡ " " " 4 " " " combined LAF_1 spleen and bone marrow cells.

9 days after the last injection. The hemagglutinating titer of the pooled sera was 1:512. Four weekly intravenous injections of combined mouse spleen and bone marrow cells were administered to donor rats in the third experiment. Each animal received a total of $1x10^8$ cells per injection. Eight days following last injection, the animals were sacrificed. The pooled sera from this group of rats similarly agglutinated LAF_1 mouse rbc with a titer of 1:512. Rat bone marrow for injection of irradiated mice was collected by the method described by Fishler, *et al.*(5) and was suspended in chilled Tyrode's solution containing 25,000 units of crystalline penicillin G per ml. A volume of 0.2 ml was injected intravenously per mouse. Spleen cell suspensions were prepared as described for preparation of mouse spleen cells. Recipient LAF_1 mice, 12 to 18 weeks of age, were exposed to a single dose of 870 r whole body X-irradiation. The X-ray source was a 250 kvp Westinghouse Therapy unit. Radiation factors were 15 ma; filter 0.5 mm Cu plus 1.0 mm Al; skin to target distance 40 inches, dose rate approximately 25 r/min measured in air. The mice were injected intravenously with the required rat cell preparation within 4 hours after irradiation. An equal number of animals was selected at random from each group and housed together. A maximum of 10 animals per cage was allowed.

Results. The results obtained from the 3 different experiments are presented in Table I. The majority of irradiated mice were dead by the 60th postirradiation day regardless of immunological status of the rat cells used for injection. A comparison between number of deaths of recipients of immunized or normal rat marrow by the chi-square method showed no significant differences between the 2 groups of animals in each experiment when tested at each 30-day interval. ($P>.1$ in all cases). A similar analysis revealed no significant increase in number of deaths when $1x10^7$ pre-immunized rat spleen cells were injected into recipient mice as compared to recipients of an equivalent amount of nonimmunized rat spleen cells.

Since the greatest number of "late deaths" occurred within the initial 60-day period, it would be expected that any enhancing effect on mortality by pre-immunized rat marrow or spleen cells would be manifested within this interval. This is based on the assumption that a graft *vs.* host immunological reaction occurs and that pre-immunization against the recipient would therefore result in an accelerated secondary response upon injection into the recipient animals. A comparison of mean

survival times at the 30- and 60-day period was made by use of the "t" test. No significant difference in mean survival time was found within each bone marrow experiment, either at the 30- or 60-day period. ($P>.1$). In 2 of these experiments mean 30-day survival time of the animals injected with pre-immunized marrow was slightly longer than that of its respective control group.

A similar comparison of mean survival time showed no significant difference between recipients of non-immune bone marrow and either $1x10^7$ normal or pre-immunized spleen cells Exp. 44 sp). It is also of interest to note that mean survival time of these animals is comparable to that of animals receiving rat bone marrow alone in the same experiment (Exp. 44). When the concentration of spleen cells injected was increased to $5x10^7$ cells, a highly significant decrease in mean survival time was found for animals receiving pre-immunized cells when compared with recipients of an equivalent amount of normal spleen.

Discussion. These results suggest that following injection of heterologous bone marrow either proliferation of the comparatively small number of immunologically active cells may be slow or proliferation of these cells may not even occur. Either of these events could then prevent the reaction of the graft against the host. These findings are in contrast to those of Cosgrove *et al.*(6) who reported enhancement of foreign bone marrow reaction following injection of pre-sensitized parental marrow into F_1 recipient mice. The basis for this variability in results may be due to the greater difference in genetic relationship between donor and recipient in the present experiments. Shekarchi *et al.*(7) have reported that acceptance of foreign grafts is directly related to genetic difference between graft and host. A higher dose of irradiation was required to impair the immune mechanism of the host against a more distantly related antigen. Since the same X-ray dose is usually employed in post-irradiation protection studies with homologous and heterologous bone marrow, a difference in initial acceptance of these grafts may result. The large amount of rat marrow cells required for "protection" as contrasted to the small amount of homologous cells needed (approximately 14-1 on wet weight basis) may be further evidence of a difference in primary acceptance of these cells and might be related to an increase in the statistical probability that a sufficient number of injected rat cells will survive to provide "protection" against acute radiation injury. In view of the small amount of lymphopoietic elements present in bone marrow, the initial destruction, by immunologic or other mechanisms of a large proportion of the injected rat cells may result in the selective colonization and subsequent proliferation of myelopoietic and erythropoietic elements only. The relative increase in circulating granulocytes(2,8) observed in rat bone marrow protected animals may be a reflection of such a selection. Further restriction on growth and proliferation of immunologically active homologous cells was suggested by the work of Boyse(9) who reported that antibody forming cells are particularly susceptible to immune attack and may therefore be eliminated sooner than other types of cells.

The inability of a supplementary injection of $1x10^7$ normal or preimmunized rat spleen cells to alter significantly mean survival time of bone marrow treated animals when compared with animals receiving bone marrow alone is further evidence for lack of extensive proliferation of heterologous immunologically active cells. In this connection, it was shown (8) that injection of bone marrow or $1x10^7$ spleen cells from rats immunized against sheep red blood cells into lethally irradiated mice did not result in transfer of antibody synthesis. However, injection of $5x10^7$ or a greater amount of spleen cells from the same immunized donors successfully transferred production of rat antibody against sheep red blood cells to the irradiated recipients. Coincidentally, a highly significant difference was observed in the present experiment between animals receiving $5x10^7$ pre-immunized rat spleen cells and $5x10^7$ normal spleen cell. In view of the short survival time of these animals, it does not appear that extensive pro-

liferation of injected cells was necessary for expression of its lethal effect.

Although growth and proliferation of heterologous (rat) myeloid cells have been successfully demonstrated in lethally irradiated mice injected with rat bone marrow, colonization by immunologically active lymphoid cells has not been conclusively shown. Gengozian *et al.*(10) and Zaalberg *et al.*(11) have reported the repopulation of lymphoid tissue of rat-mouse chimeras by donor cells. However, cell types and ability of these cells to function immunologically was not tested. Conflicting reports have also appeared regarding presence (12) or absence(13) of circulating rat type gamma globulin in rat-mouse chimeras. In view of the lack of conclusive evidence bearing on proliferation of immunologically active heterologous (rat) lymphoid cells, serious reservation must be placed on a graft *vs.* host reaction as an explanation for "late deaths" *in rat-mouse chimeras.*

Summary. A comparison was made of time and incidence of death between lethally irradiated mice injected with non-immune rat bone marrow and those injected with marrow from rats pre-immunized against recipient strain of mice. Statistical analysis of data showed no significant differences between the 2 groups of animals. Similarly, a lack of significant difference was observed with marrow-treated mice given a supplementary injection of moderate amounts of either non-immune or pre-immunized rat spleen cells. The failure of sufficient numbers of immunologically active cells to colonize and proliferate is discussed as the most probable interpretation of the results.

1. Nowell, P. C., Cole, L. J., Habermeyer, J. G., Roan, P. L., *Cancer Res.*, 1956, v16, 258.

2. Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 174.

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

4. Cole, L. J., Habermeyer, J. G., Bond, V. P., *J. Nat. Cancer Inst.*, 1955, v16, 1.

5. Fishler, M. C., Cole, L. J., Bond, V. P., Milne, W. L., *Am. J. Physiol.*, 1954, v177, 236.

6. Cosgrove, G. E., Jr., Upton, A. C., Schwartz, E. E., Congdon, C. C., Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 417.

7. Shekarchi, I. C., Makinodan, T., *ibid.*, 1959, v100, 414.

8. Chin, P. H., Silverman, M. S., *J. Immunol.*, in press.

9. Boyse, E. A., *Immunology*, 1959, v2, 170.

10. Gengozian, N., Urso, I. S., Congdon, C. C., Conger, A. D., Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 714.

11. Zaalberg, B. O., van Bekkum, D. W., *Transplantation Bull.*, 1959, v6, 91.

12. Grabar, P., Courcon, J., Illbery, P. L. T., Loutit, J. F., Merrill, J. P., *Compt. rend. Acad. Sci.*, 1957, v245, 950.

13. Gengozian, N., *J. Immunol.*, 1959, v83, 173.

Received May 24, 1960. P.S.E.B.M., 1960, v104.

Histochemical Changes in Kidneys and in Salivary Glands of Rats with Experimental Hypertension. (25892)

F. GROSS AND R. HESS (Introduced by F. F. Yonkman)

Pharmaceutical Dept., CIBA Ltd., Basle, Switzerland and Inst. of Pathology, University of Basle

Experimental hypertension produced in the rat by overdosage with cortexone acetate (DCA) and salt leads to disappearance of renin from the kidneys and to diminution of glucose-6-phosphate dehydrogenase activity in the macula densa(4). Identical changes may be observed in the unclamped kidney of rats in which hypertension is provoked by clamping one renal artery(5). These observations led us to conclude that the unclamped kidney in animals with renal hypertension is subject to similar pathogenic influences as are the kidneys of animals overdosed with DCA and salt. The fact that glucose-6-phosphate dehydrogenase (G6PD) activity is demonstrable in other structures in addition to the macula densa, *e.g.*, the small and medium ducts of salivary glands, induced us to deter-