rum from arm vein and dried finger tip blood, standard error of measurement was ± 15.5 mg cholesterol/100 ml serum. This is comparable to intra-individual variability in direct serum measurement of blood samples drawn a few days apart and is smaller than variability between serum values in casual bloods drawn at longer intervals. Significance of blood cholesterol level in prediction of risk from coronary heart disease has been under-estimated in previous studies.

1. Technical Group and Committee on Lipoproteins and Atherosclerosis, *Circulation,* 1956, v14 (no. 4, part 2), 691.

2. Dawber, T. R., Moore, F. E., Mann, G. V., *Am. J. Pub. Health,* 1957, v47 (no. 4, part 2), 4.

3. Doyle, J. T., Heslin, A. S., Hilleboe, H. E., Formel, P. F., Korns, R. F., *ibid.,* 1957, v47, 25.

4. Rivin, A. V., Yoshino, J., Shickman, M., Schjeide, O. A., *J. Am. Med. Assn.,* 1958, v166, 2108.

5. Anderson, J. T., Keys, A., *Clin. Chem.,* 1956, v2, 145.

6. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

7. Snell, F. D., Snell, C. T., *Colorimetric Methods of Analysis,* Van Nostrand, New York, 1959, 3rd Ed v2, pp279-337.

8. Mancini, M., Keys, A., PROC. SOC. EXP. BIOL. AND MED., 1960, in press.

9. Albritton, E. C., editor, *Standard Values in Blood,* A. F. Tech. Report, No. 6039, Dayton, O., 1951.

10. Keys, A., Anderson, J. T., Grande, F., *Lancet,* 1957, vii, 959.

Received March 11, 1960. P.S.E.B.M., 1960, v104.

Erythropoietic Recovery Measured by Fe_{59} Uptake in Irradiated Mice Protected with Bone Marrow.* (25872)

E. A. MIRAND, J. G. HOFFMAN AND T. C. PRENTICE

Roswell Park Memorial Inst., and Physics Dept., University of Buffalo, N. Y.

Lethal effect of whole body irradiation of mice and other mammals(1,2) is offset by intravenous grafting of nonirradiated bone marrow which may be isologous, homologous, or heterologous. It is believed that the introduced marrow cells proliferate and provide recovery by cellular repopulation or by elaborating some humoral substance which stimulates recovery of indigenous marrow. It is possible too, that transfused marrow plays a beneficial, transitory function pending recovery of indigenous marrow. Survival of animal after lethal doses of radiation depends on time of hemopoietic recovery. This has been measured in mice by recovery of the count of circulating lymphocytes and granulocytes and by recovery of hemoglobin concentration and platelet count. Recovery of hemoglobin concentration is not a satisfactory index of recovery because it is obscured by the slow downward trend of circulating survivor cells over a period of 12 to 16 days after radiation (4). Odell and Caldwell(5) found donor type erythrocytes in irradiated rats in significant numbers only after 14 to 20 days. To examine quickly the recovery of erythropoiesis we used the Fe_{59} uptake method(6) and find that recovery begins after third day post-radiation and is discernible by sixth day when isologous cells are administered. Moreover, responses elicited by homologous and heterologous cells are measurably different from one another and from the isologous case by sixth day. The method appears to distinguish the 3 genetically different cell types by the induced Fe_{59} uptake.

Methods and materials. ICR/Ha Swiss male mice, 6 weeks old weighing 20-25 g were irradiated at target distance of 30 cm, 250 kv, filter ¼ mm Cu plus 1 mm Al, with a dose of 900 R. Survival was 50% at 6 days and 0% at 12 days. Protection was given with isologous bone marrow from 6-week-old cou-

* This study supported by grants from U. S. Atomic Energy Comm., and U. S. Public Health Service. Appreciation is extended to the following Summer Fellows: William Zenosky, Douglas King and Susan Getman.

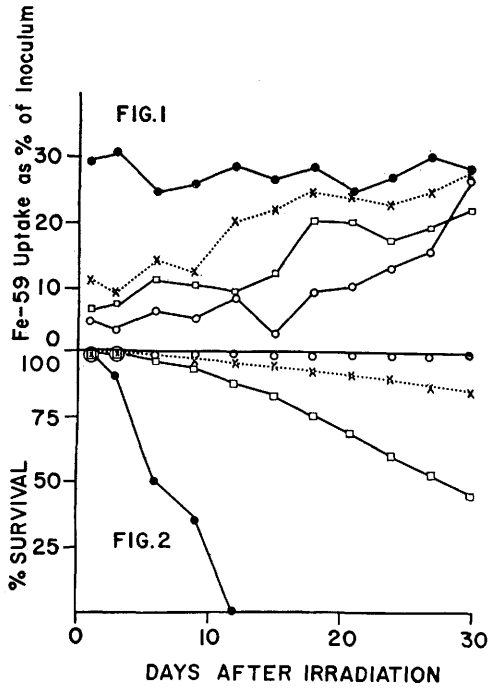

FIG. 3

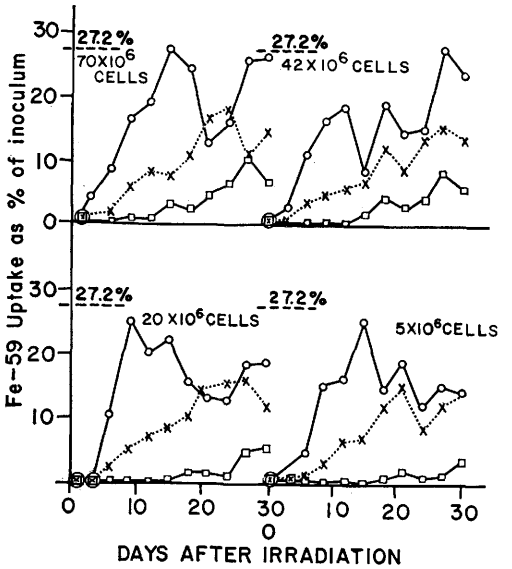

FIG. 1. Twenty-four hr Fe_{59} uptake in red blood cells of mice. Solid dots (●) show uptake in normal nonirradiated mice not receiving bone marrow cells. Other 3 curves show suppression of Fe_{59} uptake and subsequent recovery after 15 to 20 days in nonirradiated mice that received hypertransfusion of: ○ isologous, × homologous and □ heterologous marrow cells. Total cells administered were $(70 \pm 2) \times 10^6$, no suppression observed at 20 and 40×10^6 cell inocula. Cell numbers are averages. Each point represents a minimum of 6 mice and 6 determinations.

sin males; and with homologous bone marrow from DBA/1 male mice also 6 weeks old, and weighing 18-20 g. Heterologous bone marrow was taken from adult Sprague-Dawley rats (Holtzman) weighing 200-300 g. It should be emphasized that the term "isologous" in this report refers to the cousin breeding. Bone marrows were prepared by squeezing through filters the mashed tissue taken from femurs of donor mice and rats. Counts were made of viable, nucleated cells in Tyrode's Solution having 0.2% eosin. Cells stained with eosin were considered dead. Cell suspensions of known cell count were prepared and injected intravenously *via* the jugular vein within a period of 0 to 4 hours after whole body irradiation of the animal. Inoculum volume was kept constant at 0.2 cc. Assessment of erythropoietic activity was carried out as described elsewhere(6). On first, third, sixth and every third day thereafter to 30th day postradiation 6 mice were injected with 0.5 cc saline solution containing 1 μc of Fe_{59} *via* jugular vein. Uptake of Fe_{59} in circulating red blood cells was measured 24 hours after injection.

Results. Along with experimental groups of mice receiving bone marrow injections were 2 control series for each group. The first controls were mice having no radiation and no bone marrow administered. These controls totaling 156 mice established Fe_{59} uptake rate in normal mice. Solid dots in Fig. 1 give a representative series of uptake figures. From the 156 mice, average uptake rate was measured as 27.2%/24 hours, used as a basis for comparison in irradiated mice receiving mar-

FIG. 2. Survival rates of mice receiving 900 R whole body x-ray dose. Solid dots (●), mortality of mice not treated with marrow cells. Other curves show mortality in mice treated with: ×, homologous; and □, heterologous marrow cells. Circles (○) show mice receiving isologous cells had no mortality in 30 days. Cell dose was an avg of $(70 \pm 2) \times 10^6$ as in Fig. 1.

FIG. 3. Recovery of erythropoiesis as measured by Fe_{59} uptake in 24 hr interval in mice given 900 R whole body x-ray. ○, isologous; ×, homologous, and □, heterologous bone marrow cells administered intrav. postradiation. Number on each graph shows avg size of marrow cell dose. Coordinate at 27.2% uptake is avg uptake for normal nonirradiated mice not receiving marrow cells. Each point represents a minimum of 6 mice and 6 determinations.

TABLE I. Fe_{59} Uptake* as a Measure of Erythropoiesis in Swiss Male Mice Exposed to 900 R Whole Body X-Ray Dose. Uptake at various days postradiation. Marrow cells administered 0-3 hours postradiation.†

Marrow type	1 day	3 day	6 day	9 day	12 day
No marrow	.7 ± .1	.4 ± .2	.5 ± .1	.6 ± .2	.4 ± .1
Isologous	.4 ± .1	4.2 ±2.0	8.9 ±1.0	16.6 ±3.8	19.2 ±5.0
Homologous	.5 ± .2	.3 ± .1	1.9 ±3.2	5.9 ±1.0	8.3 ±1.9
Heterologous	.4 ± .1	.5 ± .3	.6 ± .3	1.0 ± .5	.8 ± .5

* Uptake in 24 hr expressed as % of inoculum.
† Cell doses were: isologous, 69 × 10^6; homologous, 71 × 10^6; heterologous, 72 × 10^6.

row cells for protection. This uptake rate in normal mice was in satisfactory agreement with earlier data(6) on Swiss male mice. No mice in this series of controls died.

The extent to which addition of marrow cells to normal, nonirradiated mice would depress normal erythropoiesis was determined in second series of controls. From earlier runs (6) it was known that hypertransfusion of red blood cells suppressed erythropoiesis as measured by Fe_{59} uptake. In 3 series of 54, 60 and 60 nonirradiated mice, each mouse received an average of 70 x 10^6 marrow cells respectively of isologous, homologous and heterologous type. Fig. 1 shows effect on Fe_{59} uptake percentages. There is a depression in the first 15 days postradiation and a subsequent recovery. The greatest depression and longest time of recovery occurred when isologous marrow was administered. There was no mortality in these controls. When 23 x 10^6 and 44 x 10^6 bone marrow cells were inoculated no depression in Fe_{59} was observed.

Protection against lethal effect of 900 R was based on previous determination of mortality(6) at this x-ray dose level. A series of 40 mice receiving x-ray without marrow established that by 12 days postradiation all animals died of severe anemia, septicemia hemorrhage and in some instances of gastrointestinal injury. Fifty percent survival was at 6 days (Fig. 2, solid dots). Measurements of Fe_{59} uptake rate are given in first line of Table I. Figures range from .7% to .4% and are much lower than those for normal or hypertransfused mice(6). The lethal effect of radiation appears to be measured by failure of Fe_{59} uptake rate since normal rates of uptake are in the range of 25 to 30%, (Fig. 1).

Survival data on irradiated mice given an average of 70 x 10^6 marrow cells of the 3 different types are shown in Fig. 2. Those receiving isologous marrow cells survived 100% after 30 days whereas homologous and heterologous cells conferred lower survivals. Homologous cells gave an 83% and heterologous cells a 45% survival after 30 days postradiation. The question as to degree of protection afforded by numbers of marrow cells was examined by administering various average numbers of cells ranging from 5 to 70 x 10^6 (Table II). Isologous cells in doses 5, 20, 40 and 70 x 10^6 afforded 100% survival at 30 days. Also, varying the cell numbers by a factor of 14 produced no significant change in survival at 30 days when homologous or heterologous cells were given. Thus, homologous cells in all doses permitted about 82% survival and heterologous cells led to a 55% survival although number of cells injected varied from 5 to 70 x 10^6. The lack of any big variation in survival with change of inoculum size suggests that there may be a threshold effect wherein a minimum number

TABLE II. Survival Data on Swiss Male Mice Given 900 R Whole Body X-Ray Dose and Treated with Bone Marrow Cells Intravenously. % survival after 30 days as function of average number of marrow cells given.

Marrow type*	5 × 10^6 cells		20 × 10^6 cells		40 × 10^6 cells		70 × 10^6 cells	
		%		%		%		%
Isologous	(59)	100	(58)	100	(59)	100	(54)	100
Homologous	(64)	81	(66)	84	(63)	81	(60)	83
Heterologous	(66)	64	(60)	61	(59)	49	(66)	45

* 40 mice given no marrow were all dead by 15th day. Parentheses contain total No. of mice used in determination of survival rates.

of cells suffices to induce the protective processes.

Discussion. The Fe_{59} uptake method for detecting hemopoiesis gives an early postradiation indication of erythropoiesis. The earliest manifestations of erythropoiesis reported hitherto are those of Odell and Caldwell(5) and deVries and Vos(7) who showed that in rats donor erythrocytes became dominant beginning at 14 days after marrow injection. Referring to Fig. 3 the 5 x 10^6 cell doses of isologous, homologous and heterologous produced responses in uptake rate detectable at 3 to 6 days postradiation. At 6 days, uptake for isologous cell treatment was 8.9% and those for homologous and heterologous cells were 1.2 and .5% respectively. Table I gives more detailed figures for responses at the 70 x 10^6 cell dose levels. Mice without marrow cell treatment had an uptake less than 1% as pointed out above (data in Table I, first line).

The isologous cells produced an uptake of 4.2% by the 3rd day, a figure readily measurable. In this respect, the Fe_{59} uptake method compared favorably with the lymphocyte count method(4) which is sensitive since these cell types of the recipient are removed quickly from circulation after radiation. These results are consistent with the view that in supralethally irradiated mice the isologous cells provide erythropoiesis. Uptake of Fe_{59} thus gives a sensitive and quick measure of erythropoiesis in presence of high levels of hemoglobin concentration at early times postradiation.

The significant differences seen between the curves for various injected groups points out that isologous bone marrow populates sooner than homologous and heterologous bone marrow. Factors for this are not definitely understood, however, isologous bone marrow certainly stands a better chance for cellular repopulation in a more genetically related irradiated host.

The mortality data do not correlate closely with the data on erythropoietic activity as measured by Fe_{59} uptake rate, (Fig. 3). The ordinate at 27.2% shows normal uptake in nonirradiated mice without marrow cell injection. Uptake in mice given isologous cells shows a rapid return to normal rates in all cases, by the 15th day, which is independent of cell numbers administered. This is also the result for mice given homologous cells, except that where the lowest cell dose was given (5 x 10^6 cells) initial recovery in the first 9 days is slightly slower than at higher cell number doses. Mice receiving less than 40 x 10^6 heterologous cells have a much lower recovery of uptake than do those receiving doses larger than 40 x 10^6, (Fig. 3). The difference, however, did not appear to affect survival figures, (Table II). It is, indeed, hard to explain the 64% survival rate, (Table II), on the basis of uptake data for a cell dose of 5 x 10^6.

The mortality data of Table II bear out the established genetic relationship between donor and recipient described by Trentin(2) and Uphoff and Law(8). The heterologous marrow cells being farther removed genetically from the recipient afforded less survival. The trends of the curves in Fig. 3 fit the concept that isologous cells are genetically closer to the recipient than are homologous cells from DBA/1 mice or the heterologous cells from rats. Iron uptake rates are clearly greatest for the isologous cells: homologous cells stimulate erythropoiesis greater than do heterologous cells but less than isologous cells. The consistency of genetic behavior as cell type varies supports the inference that protection conferred by the marrow cells is not due to stimulation of erythropoiesis alone. This is clearly indicated in the heterologous case wherein the Fe_{59} uptake is very low for the first 15 days postradiation. Hence survival cannot be due to erythropoiesis alone. If protection depends on cellular elements in circulation then "the reticulo-endothelial and myelogenous systems" apparently are involved in protection afforded by the marrow graft. For example, the early appearance of circulating leucocytes as shown by Odell and Caldwell(5), and the effect of cell and spleen extracts, as shown by Goldfeder and Clarke (9), suggest other supporting mechanisms necessary for survival.

Summary. 24 hour uptake of Fe_{59} was

used as quick measure of erythropoiesis in Swiss male mice. In normal nonirradiated mice, iron uptake is not affected by intravenous injection of isologous, homologous and heterologous bone marrow cells except for inocula greater than 40 x 10^6 cells. Injection of these cell types after 900 R whole body x-radiation increases Fe_{59} uptake rates in the order: heterologous, homologous, isologous. Protection against lethal radiation effect as measured by survival and Fe_{59} uptake depended on genetic relationship between bone marrow donor and recipient.

1. Doherty, D. G., Congdon, C. C., Makinodan, T., Hollaender, A. *Radiation, Biology and Medicine,* Reading, Mass., 1958, Chap. 15, 369.

2. Trentin, J. J., *J. Nat. Cancer Inst.,* 1959, v22, 219.

3. Gengozian, N., Makinodan, T., Congdon, C. C., Owen, R. D., *Proc. Nat. Acad. Sci.,* 1958, v44, 560.

4. Smith, W. W., Marston, R. Q., Cornfield, J., *Blood,* 1959, v14, 737.

5. Odell, T. T., Jr., Caldwell, B. C., *J. Nat. Cancer Inst.,* 1958, v20, 851.

6. Mirand, E. A., Hoffman, J. G., Prentice, T. C., Reinhard, M. C., *Exp. Med. Surg.,* 1958, v16, 258.

7. de Vries, M. J., Vos, O., *J. Nat. Cancer Inst.,* 1959, v23, 1403.

8. Uphoff, D. E., Law, L. W., *ibid.,* 1959, v22, 229.

9. Goldfeder, A., Clarke, G. E., *Rad. Res.,* 1957, v6, 318.

Received April 4, 1960. P.S.E.B.M., 1960, v104.

Effects of Pyridoxine and Desoxypyridoxine on Magnesium Metabolism in the Rabbit.* (25873)

JERRY K. AIKAWA
(With technical assistance of Jacqueline Z. Reardon and Dale R. Harms)
Dept. of Medicine, University of Colorado School of Medicine, Denver.

Factors regulating metabolism of magnesium are obscure. Development of a relatively simple and reliable chemical method for determination of magnesium in biological material, and availability of radioactive isotope of magnesium, Mg^{28}, have made possible a restudy of the entire problem of magnesium metabolism. Studies of plasma clearance and tissue uptake of Mg^{28} in experimental animals (1) and in human beings(2) have been reported. Changes in carbohydrate metabolism alter rate of uptake of Mg^{28} by various tissues(3). The effects of cortisone acetate have also been determined (unpublished). These studies suggested that tissue uptake of Mg^{28} was considerably slower than that of another intracellular ion, potassium, and that cellular uptake of magnesium might be associated with anabolic activities of cells. If this hypothesis is correct, then any factor altering metabolism of protein might affect that of magnesium. Pyridoxine deficiency alters protein metabolism and produces changes in enzymatic reactions(4); pyridoxine also influences magnesium metabolism(5). Our experiments were undertaken to determine effects of pyridoxine and its antagonist, desoxypyridoxine, on magnesium metabolism in the rabbit. Mg^{28} was used to measure exchangeable body content of this ion and its relative tissue uptake.

Material and methods. Forty-seven normal adult male rabbits weighing between 1.6 and 2.5 kg were kept in individual stainless steel metabolism cages and fed a stock diet of compressed pellets. Tap water was given *ad lib. Pyridoxine* hydrochloride and *desoxypyridoxine* hydrochloride were dissolved in distilled water, neutralized with 1 N NaOH, and made to concentration of 40 mg/ml. *Exp. I and II.* External balance studies for magnesium were performed daily at least 11 days in 2 groups of 8 rabbits each. During first 2 days, baseline studies were done. Starting on third day and continuing 8 days, each animal in

* This work supported by contract between University and U. S. Atomic Energy Comm. and by grant from Colorado Heart Assn.