To the Graduate Council:

I am submitting herewith a thesis written by Stephen J. Layendecker entitled "The Relative Roles of the Spleen and Bone Marrow in Platelet Production." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Radiation Biology.

Ted P. McDonald, Major Professor

We have read this thesis
and recommend its acceptance:

Howard T. Allen

T. T. Odell

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

THE RELATIVE ROLES OF THE SPLEEN AND BONE MARROW IN PLATELET PRODUCTION

A Thesis

Presented for the

Master of Science

Degree

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Stephen J. Layendecker

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ABSTRACT

The relative importance of the spleen and of the bone marrow in platelet production is not known for mice. In an attempt to delineate these roles in mice, the spleen was removed by surgical excision or the bone marrow was ablated with an injection of strontium-89 (^{89}Sr). Platelet production in these mice was compared with that in untreated mice, ^{88}Sr (nonradioactive strontium)-treated mice, sham-splenectomized mice, and whole-body irradiated mice. Platelet production and platelet counts in ^{89}Sr marrow-ablated mice were similar to platelet production and platelet counts in whole-body irradiated mice, as were RBC counts, WBC counts, and hematocrit values, until the spleen became larger (150% of normal by day 9) and apparently began to produce platelets to compensate for the loss of the bone marrow. Splenectomy did not affect the capability of mice to maintain normal platelet production and platelet counts, or RBC counts, WBC counts, and hematocrit values. Therefore, under normal conditions the spleen contributes little or nothing to platelet production in mice, but after bone marrow removal, platelet production by the spleen may occur as a compensatory mechanism. The platelets produced by the compensating spleen resemble in size those normally produced by the marrow after thrombocytopenia, with above average sizes. After splenectomy, platelets were of decreasing average size indicating that older, smaller platelets were being retained in the circulation since the spleen was no longer present to serve as a platelet trapper.

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INTRODUCTION

Strontium-89 (^{89}Sr) and splenectomy procedures have been used to study the relative roles of the spleen and bone marrow in red blood cell production (1-2), therapeutic efficiency of colony forming units (CFUs) (3), and stem-cell dynamics (4). However, these techniques have not been used to study the relationships of the spleen and the bone marrow for platelet production. The relative roles of these organs in thrombocytopoiesis in normal mice are not understood. In these studies, bone marrow was ablated by injection of strontium-89 or the spleen was removed by surgical excision and the treated mice were examined for platelet production. The results strongly suggest that the spleen produces few (if any) platelets in normal mice but that after marrow ablation with ^{89}Sr, the spleen becomes enlarged and appears to compensate for the loss of the marrow.

CHAPTER I

MATERIALS AND METHODS

Male C3H mice, 8-10 weeks old and weighing approximately 22.5 grams, were used throughout and were housed ten to a cage with food and water administered ad libitum.

Whole-body irradiation was delivered as previously described (5) by a G. E. Maximar III X-ray unit. The mice were exposed to a total of 750R at a rate of 80 R/min by 225 kvp, 15 mA X-rays. Target-to-object distance was 50 centimeters. During the entire treatment, the animals were rotated axially in plastic holders with a ten-mouse capacity at 8.5 rev/min. A 1.0 mm Al filter was used for all exposures.

Marrow ablation was caused by ^{89}Sr injections; ^{89}Sr was injected in the form of aqueous strontium chloride ($SrCl_2$). Strontium-89 is a bone-seeking, beta emitter which rapidly localizes in the bone within 24 hours after injection (1, 2) and causes a minimal exposure to other tissues. The $^{89}SrCl_2$ was diluted into saline to a concentration of 400 microCuries per ml and injected intraperitoneally (i.p.) at a dose of 4 microCuries per gram of body weight (2, 4). This concentration of isotope will prevent any significant production of hematopoietic cells by the marrow for at least 30 days after injection (6). The ^{89}Sr was obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee, and from Amersham Radiochemical Center, Amersham, England.

Splenectomy was performed under methoxyflurane anesthesia. A 1.5 cm incision was made along the lateral midline through the skin and

a 0.6 cm incision was then made through the peritoneum. The spleen was removed at the pedicle to insure that all splenic material had been removed. The peritoneum was sutured with a 4-0 silk and the skin was sealed with stainless steel wound clips. The survival rate was 97%.

Untreated animals, ^{88}Sr (cold strontium)-injected animals, and sham-splenectomized animals were used as controls. Combinations of treatments, also used as controls, included the use of splenectomy/^{89}Sr injection and splenectomy/irradiation. Experimentation of control mice was identical to the animals treated with ^{89}Sr, splenectomy, or irradiation.

Immediately before sacrifice, blood for peripheral platelet counts, red blood cell (RBC) counts, white blood cell (WBC) counts, and for determination of hematocrit values was obtained from the retro-orbital sinus. These measurements were performed by standard techniques (Appendix A).

For the determination of platelet sizes and percent ^{35}S-incorporation into platelets, blood was obtained via heart puncture following a 0.5 ml i.p. injection of a heparin-Nembutal solution (1.0 ml heparin, 1000 U.S.P. units; 1.0 ml Nembutal, 50 mg and 8 ml saline). The mice were bled from the heart into syringes containing 1.0 ml of 1.0% ethylenediaminetetraacetic acid (EDTA) solution, and the blood was expressed into plastic tubes (7). An average of 0.5 ml of blood was obtained from each mouse.

For platelet sizing, the blood was diluted to a volume of 2 ml by adding additional 1.0% EDTA solution, and centrifuged for 4.5 minutes at

150 g at 22°C. The resulting platelet-rich plasma was then used as a source of platelets for sizing by a procedure previously outlined (7, 8).

Average platelet size (on a logarithmic scale) for each mouse was determined with an Electrozone/Celloscope (Particle Data), a computerized instrument with a logarithmic amplifier (Appendix B). Instrument settings were log 10 (logarithmic span of ≃ 10 volume doublings or ≃ 10:1 in diameter), current 6.5 (≃ 1 milliamp), and gain 3.0 (≃ 160 X). The instruments were set to cover a particle size from .70 to 36.28 cubic microns. Data were accumulated in 149 of the 256 channels. Calibration was frequently verified with latex particles of known size (4.32 m^3 volume; Dow Chemical).

Platelet production rates were determined using the index of percent ^{35}S-incorporation into platelets as previously described (9). Twenty-four hours before sacrifice, mice were injected intravenously (i.v.) with 30 μCi of $Na_2{}^{35}SO_4$ in 0.5 ml of saline. The percentage of the injected dose incorporated into circulating platelets was determined from the platelet-rich plasma used for platelet sizing. The platelet-rich plasma was subjected to four centrifugation procedures (15 minutes at 600 g at 22°C). Each centrifugation yielded a platelet pellet. The platelets were washed after the first centrifugation with 0.6 ml of 1.0% ammonium oxalate, after the second with 0.5 ml of 1.0% ammonium oxalate, and after the third with 1.5 ml saline. After the last centrifugation, suspension counts were made and the radioactivity was determined (Appendix C).

Spleen weights from some mice were measured after ^{89}Sr injection and irradiation. After the cardiac puncture, the spleens were removed and weighed within 45 minutes of removal on a Mettler balance. (Spleens

were kept in a moist chamber until weighed.) All excess fat and other tissues were removed from the spleen prior to weighing. The spleens were weighed in groups, not individually, and the average weights were determined from the results of two groups of 3 mice each for each day.

Blood volume determinations on splenectomized mice were made using a standard ^{59}Fe-labelled red blood cell dilution technique (10, 11), (Appendix D).

Student's *t* test was used to analyze the data.

CHAPTER II

RESULTS

Results of RBC counts after irradiation, ^{89}Sr injection, and splenectomy are shown in Table 1 (Appendix E). RBC counts in irradiated mice immediately decreased to below normal levels and this was sustained for the duration of the experiment. Strontium-89 marrow-ablated mice also showed a decrease to below normal levels although the response was not as immediate. The RBC counts for ^{89}Sr-treated mice were significantly below normal for all days tested except days 2, 7, and 10. The RBC counts of splenectomized mice were at normal levels except for days 2, 4, 6, and 11. The counts of sham-splenectomized and ^{88}Sr-treated mice remained at normal levels throughout the experiment.

The results of WBC counts for the same mice as presented in Table 1 are depicted in Table 2 (Appendix E). WBC counts for irradiated and marrow-ablated mice were significantly ($P < 0.0005$) below normal for each day tested. For splenectomized mice, the counts were significantly ($P < 0.0005$) above normal for each day tested except on day 1. The WBC counts for sham-splenectomized mice and ^{88}Sr-treated mice remained at normal levels throughout the experiment.

Results of hematocrit values for the same mice are shown in Table 3 (Appendix E). Hematocrit values of irradiated mice were normal on days 1 and 2 but dropped to below normal values on day 3 and remained there for the remainder of the experiment. The patterns in strontium-treated mice were similar to those of irradiated mice on days 1-6 but then hematocrit values for ^{89}Sr-treated mice returned to normal by day 7

and subsequently remained normal. Hematocrit values for splenectomized mice were normal except on days 1, 2, and 4 when they were below normal. Hematocrit values for sham-splenectomized and ^{88}Sr-treated mice were normal throughout the experiment. Bleeding in sham-splenectomized mice was considerably less than in splenectomized mice.

The effects of whole-body irradiation, ^{89}Sr marrow-ablation and splenectomy on the peripheral platelet counts of mice are presented in Figure 1 (Appendix F). Peripheral platelet counts showed an almost linear increase with time in splenectomized mice; levels significantly different from controls were reached by day 4 ($P < 0.005$) and continued to increase until day 6 ($P < 0.0005$). After day 6 platelet numbers decreased to near normal levels by days 7-11. Peripheral platelet counts of marrow-ablated mice and irradiated mice showed similar initial patterns; both treatments led to a decline in numbers of platelets with values significantly ($P < 0.0005$) below normal by day 4 or 5. However, unlike the irradiated mice, the platelet counts of marrow-ablated mice had leveled off by day 8 and thereafter showed evidence of increasing, indicating that another site must be compensating for the loss of marrow thrombocytopoiesis. (All mice subjected to irradiation were dead by day 9). Peripheral platelet counts of ^{88}Sr (nonradioactive strontium)-injected mice and sham-splenectomized mice were not significantly different from normal.

In Figure 2 (Appendix F), peripheral platelet counts of mice subjected to combined treatments of splenectomy/^{89}Sr and splenectomy/irradiation are compared to those of irradiated mice. Platelet counts declined similarly after all three treatments, indicating that irradiation prevented both the spleen and bone marrow from producing platelets.

The platelet counts also showed that extramedullary sites were not producing significant numbers of platelets in mice treated with ^{89}Sr or mice that had been splenectomized.

Platelet production as measured by percent ^{35}S-incorporation into platelets is depicted in Figure 3 (Appendix F). Splenectomized mice (Panel A) showed an increase to higher than normal levels on days 3-6, and a decrease thereafter with a one-day excursion to below normal levels on day 9. Percent ^{35}S-incorporation into platelets of ^{89}Sr marrow-ablated mice (Panel B) was unexpectedly higher than normal on days 1-3; but then the values declined to below normal for days 5-8. After day 8 percent ^{35}S-incorporation into platelets had returned to normal levels. Percent ^{35}S-incorporation into platelets of irradiated mice (Panel C) remained significantly below normal levels for the duration of the experiment except on day 2. Percent ^{35}S-incorporation into platelets of ^{88}Sr-treated mice (Panel B) shows increased levels on days 1-6 and thereafter a return to normal by day 12.

The results of platelet sizing experiments are shown in Figure 4 (Appendix F). Platelets from splenectomized mice (Panel A) decreased in average size by day 4 and thereafter remained below normal; values were significantly ($P < 0.005$, $p < 0.0005$) below normal on days 4, 7, and 8. In agreement with previous work (11), platelet sizes remained below normal for the duration of the experiment. Platelet sizes in ^{89}Sr marrow-ablated mice (Panel B) showed a period of no change until day 7, except for day 3 when there was a small but significant increase noted. After day 7, the average platelet sizes increased significantly ($P < 0.0005$) and remained elevated throughout the remainder of the experiment. Platelet sizes of irradiated mice (Panel C) were slightly

below normal for much of the experiment but, as was observed in platelet counts, there were similarities between the irradiated mice and the marrow-ablated mice until day 7. After day 7, platelet sizes of irradiated mice did not increase to the extent that occurred in ^{89}Sr-treated mice but, rather, remained at normal levels.

Although not shown, blood volumes of splenectomized mice did not significantly differ from the blood volumes of untreated mice (5.98 ± 0.15 of body weight on days 1-14).

Average spleen weights from irradiated and ^{89}Sr-treated mice are shown in Figure 5 (Appendix F). The data show an increase to 150% of normal values by day 10 for spleens of ^{89}Sr-treated mice. Spleens of irradiated mice showed a decrease to 25% of normal values by day 10.

CHAPTER III

DISCUSSION OF RESULTS

In a previous study (2), platelet counts were reduced after ^{89}Sr-injection but platelet numbers recovered to normal levels in Cornet mice 14 days later. However, platelet production rates were not measured after ^{89}Sr-marrow ablation. To determine the relative contributions of the spleen and bone marrow in platelet production, C3H mice were subjected to ^{89}Sr-injection to cause marrow ablation, splenectomized, or exposed to whole-body irradiation. The results of these studies showed reduced platelet counts and decreased percent ^{35}S-incorporation into platelets after ^{89}Sr-injection and whole-body irradiation, but little change in these parameters was observed after splenectomy. The results indicated that the spleen does not normally contribute significantly to thrombocytopoiesis, but may perform a compensatory role in platelet production after the loss of the bone marrow. In addition, thrombocytosis and a decrease in the mean size of platelets after splenectomy indicate that the spleen may alter platelet counts of mice by platelet trapping and/or suppression of bone marrow thrombocytopoiesis.

Both ^{89}Sr-treatment (2) and whole-body irradiation (12-15) have been shown to reduce platelet counts of mice. Irradiation causes aplasia of both bone marrow and spleen and thereby reduces the megakaryocyte population leading to thrombocytopenia (16). ^{89}Sr selectively causes bone marrow aplasia without affecting the spleen. As shown in Figure 1 (Appendix F), platelet counts of irradiated and ^{89}Sr-treated mice were reduced in a similar pattern as were RBC counts (Table 1,

Appendix E), WBC counts (Table 2, Appendix E), and hematocrit values (Table 3, Appendix E). The reduction in platelet counts with time after ^{89}Sr-treatment and irradiation, along with the fact that platelet counts of splenectomized mice were not reduced, suggests that in normal mice the spleen is not producing platelets to any significant degree. The ^{89}Sr-treated mice showed a slower reduction in platelet counts with time than irradiated mice, but this finding may be explained by the incorporation time of the radioisotope. In addition, the hypothesis that the spleen does not normally contribute significantly to platelet production is strengthened by the pattern of platelet counts of mice that were treated with ^{89}Sr-injection/splenectomy (Figure 2, Appendix F). ^{89}Sr-injection/splenectomy did not cause a faster reduction in platelet counts than ^{89}Sr-treatment alone (Figure 1, Appendix F). In fact, mice given whole-body irradiation, splenectomy/^{89}Sr-treatment, and splenectomy/irradiation all showed about the same decrease in platelet counts with time. Although the spleen contains a large number of megakaryocytes (17), the present data suggest that these megakaryocytes do not normally produce platelets.

In addition to platelet counts, the results of measuring the percent ^{35}S-incorporation into platelets, as an index of platelet production, supports the above hypothesis. Both irradiated and ^{89}Sr-treated mice (Figure 3, Appendix F) showed a reduction in isotope incorporation into platelets with time, but platelet production in splenectomized mice was not reduced. This finding, along with the reduced platelet counts of ^{89}Sr-treated mice, indicates that normally, platelet production is almost entirely by the bone marrow.

The elevated ^{35}S-incorporation into platelets of ^{89}Sr-treated mice on days 1-3 was an unexpected finding (Figure 3, Appendix F). However, similar results were obtained in mice after injection of non-radioactive strontium (^{88}Sr), indicating that the immediate and highly significant increase in ^{35}S-incorporation into platelets was the result of toxic or chemical effects of strontium and not to radioactivity of the ^{89}Sr. Also, since platelet counts were not elevated after Sr-treatment, these high ^{35}S-incorporation values probably do not represent increased platelet production patterns. It is tempting, therefore, to speculate that the platelet acting as a phagocyte is responsible for taking up additional ^{35}S. Strontium chloride could react with $Na_2{}^{35}SO_4$ to yield strontium sulfate which would drastically change its solubilities. The precipitated $^{89}Sr^{35}SO_4$ could then be phagocytized by platelets resulting in increased ^{35}S-incorporation into platelets of mice. Platelet phagocytosis of submicroscopic particles such as cobalt and carbon are well known (18), but the ability of platelets to phagocytize precipitates such as $SrSO_4$ is not known. Phagocytosis of precipitates by platelets would explain the findings of the present report, but, obviously, data to support this supposition are lacking and the reason for elevated ^{35}S-incorporation into platelets following Sr-treatment awaits further experimentation.

Measurement of platelet sizes may also be a technique for determination of platelet production by the spleen and bone marrow. Circulating platelets represent a heterogeneous population of different size platelets, and young platelets are known to be larger than old platelets (19-22). Normally, a platelet population has a consistent mean size. A larger mean platelet size is consistent with increased

platelet production while a decreasing mean platelet size is associated with decreased platelet production (23). In irradiated mice and mice given ^{89}Sr, mean platelet size remained normal on days 1-7 which is consistent with a lack of increased platelet production. Decreasing mean platelet sizes in splenectomized mice, which also have increased percent ^{35}S-incorporation into platelets, appears to be related to removal of splenic function. However, increased platelet sizes on days 8-10 (Figure 4, Appendix F) after ^{89}Sr-treatment is consistent with the hypothesis of increased platelet production by the spleen.

Although these experiments show that the spleen does not normally contribute significantly to platelet production, other workers (1-4, 6) have shown that under stressful situations the spleen may compensate for the loss of the bone marrow by producing platelets and other blood cells. Gurney et al. (2) have shown that this compensatory mechanism is associated with splenomegaly in mice after injection of 6 μCi/g of body weight of ^{89}Sr. In agreement with these workers, the present work indicates that the spleen performs a compensatory action after bone marrow ablation. Platelet sizes (Figure 4, Appendix F), platelet production (Figure 3, Appendix F), and platelet counts (Figure 1, Appendix F) in ^{89}Sr marrow-ablated mice began to recover by day 9-10. This recovery was accompanied by a dramatic increase in spleen weight (Figure 5, Appendix F). Splenomegaly was evident by day 2 and continued throughout the experiment, with spleen weights averaging 150% of normal by day 10. The spleen weights found in the present work were consistent with those found by Gurney et al. (2). Although spleen weights increased rapidly, platelet production in ^{89}Sr-treated mice did not return to normal until the spleen was approximately 1½ times its

original size. As expected, compensatory action of the spleen was absent in irradiated mice; both the rapid decrease in spleen weights and the continued decrease in platelet counts confirm that the spleen had been eliminated as a site of platelet production in these mice. The liver and other extramedullary sites can be eliminated as possible areas of platelet production because of similarities in platelet counts between ^{89}Sr-injected/splenectomized and whole-body irradiated mice (Figure 2, Appendix F). This finding is consistent with the results of Adler et al. (24) who found that the adult liver is not an active site of hematopoiesis. Bone marrow repopulation can also be ruled out as a factor contributing to recovery since there is no significant hematopoietic activity in the bone marrow for 30 days (6) after ^{89}Sr-treatment. The results of the present work, therefore, indicate that normally, in mice the bone marrow is the site of production for almost all platelets, but after the marrow is removed, the spleen enlarges and begins to produce platelets.

Previously, it was shown that the spleen may influence platelet production or platelet counts by serving as a platelet trapper or graveyard (25), while other workers (26, 27) have suggested that the spleen may act to regulate bone marrow activity. The results of the present work are not conclusive for either of these hypotheses. The role of the spleen as a platelet trapper or graveyard is analogous to the role suggested for the spleen as a RBC graveyard by Crosby (28). In this model, the spleen functions to remove old or damaged platelets. Splenectomy should therefore, result in increased platelet counts with an accompanying decrease in the mean size of the platelet population, since platelet size decreases with age. A finding of decreased platelet

mean sizes after splenectomy, as was found herein, would be evidence that older, smaller platelets are being retained in the circulation. Increased platelet counts after splenectomy have been previously described (29). The present work, therefore, supports the "graveyard" hypothesis. Splenectomized mice showed increasing platelet counts and a decreasing average platelet size with time in agreement with previous work (11). The possible decrease in platelet counts on days 1 and 2 after splenectomy may be attributed to consumption of platelets at the site of surgery, as other studies have suggested (30, 31).

Although an increase in platelet counts of mice after splenectomy supports the "graveyard" hypothesis, the thrombocytosis along with elevated platelet production (Figure 3, Appendix F) after splenectomy seems to support the notion that the spleen can release chemicals that suppress bone marrow activity. It has been postulated (27, 32) that the spleen produces a humoral factor that suppresses bone marrow activity, and removal of the spleen may cause increased platelet counts through increased platelet production (Figure 3, Appendix F). A temporary deregulation of the bone marrow to increase CFU production has been reported (4). It appears that both platelet trapping and release of humoral factors to suppress bone marrow activity may be roles of the spleen, with both mechanisms contributing to increased platelet counts in splenectomized mice.

The work herein shows that the spleen does not normally contribute a significant proportion of platelets to the circulation, but may do so under certain circumstances such as bone marrow ablation. Preliminary data on mice that have received ^{89}Sr injection and platelet antisera also suggest that the spleen may produce platelets in response to

thrombocytopenia. The present work supports the role of the spleen as a platelet "graveyard," but the work also suggests that the spleen may produce inhibitory chemicals that suppress bone marrow activity.

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APPENDICES

APPENDIX A

BLOOD MEASUREMENTS

Peripheral platelet counts were determined using phase microscopy. This procedure involves dilution of a blood sample with 1.0% ammonium oxalate in a red blood cell pipette, placement of the sample on a hemacytometer (American Optical), 1/10 mm deep, and direct counting of the platelets.

Red blood cell (RBC) counts and white blood cell (WBC) counts were determined on a Coulter Counter, model Zf. A blood sample was taken with a .02 ml Accupette (Scientific Products) and expressed into 10 ml of Isoton solution (1:500 dilution). Of this solution, 0.1 ml was then removed and expressed into Isoton to obtain a 1:50,000 dilution and then counted for RBC. To the remainder of the whole blood solution was added 3 drops of Hematall LA-Hgb reagent (Fisher) and counted for WBC. The RBC count x 100 equals RBC/cu. mm. The WBC count equals WBC per cu. mm.

Hematocrits were determined by the use of heparinized microhematocrit tubes using a Micro Hematocrit Centrifuge, model MB, International Equipment Company (IEC) and the Micro-Capillary Reader (IEC).

APPENDIX B

PLATELET SIZING

For determination of platelet sizes, platelets were diluted with filtered saline and the total number of particles was adjusted to between 1.5 - 2.0 x 10^4/100 microliters of sample to maintain a coincidence error of less than 1% when used with the 48 micrometer orifice tube in the instrument. The channel numbers are proportional to the logarithms of size expressed as volume. Platelet sizes actually represented the geometric mean of the sample. All platelet sizes were determined at 22°C, and were analyzed within one hour of removal from the host.

APPENDIX C

PERCENT ^{35}S-INCORPORATION INTO PLATELETS

$$\text{Percent } ^{35}\text{S-incorporation into platelet} = \frac{\dfrac{\text{cpm of platelet suspension}}{\text{count of platelet suspension}} \times \text{BW} \times 0.063 \times \text{peripheral platelet count/ml}}{\text{cpm injected}} \times 100$$

Counts per minute of suspension are determined by a Packard Liquid Scintillation Spectrometer. Platelet counts of the suspension were determined by the ElectroZone/Celloscope (Particle Data). The body weight x 0.063 represents the blood volume of the mouse. The peripheral platelet count/ml is taken from the count by phase microscopy. The counts per minute injected is determined from the experimental isotope standard as counted by the scintillation counter.

APPENDIX D

BLOOD VOLUME DETERMINATION

To determine blood volumes 15-20 mice served as erythrocyte donors and were given a single injection (i.p.) of 0.5 microCuries of ^{59}Fe (ferric citrate). Forty-eight hours later the mice were sacrificed and approximately 6 ml of blood were collected into a tube containing 1 ml of sodium citrate. The blood was centrifuged, and the citrate-plasma portion was removed. The red cells were then mixed with saline to the original blood volume, 0.1 ml of this solution was then injected (i.v.) into mice. These mice were bled 15-20 minutes later and 100 microliters of this blood were diluted into 2 ml of water and counted for radioactivity. Blood volumes were calculated using the following formula:

$$\left[\frac{(\text{cpm injected})}{(\text{cpm sample} - \text{cpm background})} \times 0.1\right] - 0.1 \times \frac{100}{BW} = \text{Blood volume}$$

APPENDIX E

TABLE 1

EFFECTS OF WHOLE-BODY IRRADIATION, STRONTIUM-89, AND SPLENECTOMY ON RED BLOOD CELL COUNTS OF MICE WITH TIME

Days After Treatment	Whole-Body Irradiation RBC x $10^{-5}/mm^3$	Strontium-89 RBC x $10^{-5}/mm^3$	Splenectomy RBC x $10^{-5}/mm^3$
0	75.7 ± 0.9 (49)	-	-
1	66.6 ± 1.4** (11)	77.5 ± 2.3 (3)	73.4 ± 2.1 (9)
2	67.7 ± 1.5** (12)	64.5 ± 4.1* (3)	66.3 ± 1.1** (9)
3	69.9 ± 0.9* (10)	60.5 ± 2.5** (9)	67.9 ± 4.5 (5)
4	62.3 ± 1.2** (11)	57.7 ± 2.7** (9)	64.1 ± 3.5** (10)
5	68.9 ± 2.3* (9)	66.0 ± 0.7** (9)	68.4 ± 3.8 (4)
6	62.5 ± 0.9** (9)	58.8 ± 2.9** (9)	63.8 ± 1.4* (3)
7	56.6 ± 1.4** (12)	73.3 ± 1.6 (9)	72.9 ± 1.6 (7)
8	55.4 ± 1.0** (9)	65.3 ± 1.4** (9)	68.5 ± 3.1 (4)
9	48.4 ± 2.5** (5)	59.7 ± 1.4** (9)	70.5 ± 1.5 (4)
10	28.8 ± 1.4** (2)	70.2 ± 2.4 (9)	-
11	-	-	65.6 ± 3.0* (4)
14	-	-	76.8 ± 1.6 (4)

Each value is the mean ± the SE.

*Significantly different from the untreated control mice: $P < 0.005$.

**Significantly different from the untreated control mice: $P < 0.0005$.

( ) indicates number of mice used.

TABLE 2

EFFECTS OF WHOLE-BODY IRRADIATION, STRONTIUM-89, AND SPLENECTOMY ON WHITE BLOOD CELL COUNTS OF MICE WITH TIME

Days After Treatment	Whole-Body Irradiation WBC x $10^{-2}/mm^3$	Strontium-89 WBC x $10^{-2}/mm^3$	Splenectomy WBC x $10^{-2}/mm^3$
0	91.6 ± 2.0 (51)	-	-
1	27.1 ± 2.3** (11)	48.4 ± 7.7** (2)	79.5 ± 9.4 (9)
2	37.6 ± 1.4** (12)	55.4 ± 5.3** (3)	118.3 ± 5.8** (9)
3	8.5 ± 3.6** (10)	32.6 ± 2.2** (9)	117.1 ± 7.6** (5)
4	6.2 ± 1.2** (11)	29.4 ± 3.5** (9)	125.6 ± 10.2** (10)
5	5.7 ± 1.2** (9)	25.7 ± 1.4** (9)	145.0 ± 17.8** (4)
6	2.5 ± 0.4** (9)	41.1 ± 6.2** (9)	148.7 ± 9.6** (3)
7	1.8 ± 0.1** (12)	36.8 ± 4.6** (9)	118.9 ± 7.7** (7)
8	3.2 ± 0.2** (8)	25.5 ± 2.3** (9)	114.4 ± 10.5* (4)
9	2.3 ± 0.6** (5)	35.8 ± 4.3** (9)	122.7 ± 12.2** (4)
10	2.7 ± 0.9** (2)	31.3 ± 1.6** (9)	-
11	-	-	118.7 ± 11.8* (4)
14	-	-	131.0 ± 5.1** (4)

Each value is the mean ± the SE.

*Significantly different from untreated control mice: $P < 0.005$.

**Significantly different from untreated control mice: $P < 0.0005$.

( ) Indicates number of mice used.

TABLE 3

EFFECTS OF WHOLE-BODY IRRADIATION, STRONTIUM-89, AND SPLENECTOMY ON HEMATOCRIT VALUES OF MICE WITH TIME

Days After Treatment	Whole-Body Irradiation %	Strontium-89 %	Splenectomy %
0	45.8 ± 0.4 (45)	-	-
1	44.7 ± 0.6 (8)	43.7 ± 0.3 (3)	41.6 ± 1.7* (5)
2	43.1 ± 0.6 (8)	42.0 ± 0.6 (3)	42.3 ± 0.3* (4)
3	42.7 ± 0.6* (10)	40.8 ± 0.6** (9)	44.0 ± 0.9 (4)
4	40.0 ± 0.6** (10)	40.9 ± 0.6** (9)	41.5 ± 1.3** (10)
5	39.9 ± 0.6** (8)	42.7 ± 0.4* (9)	43.8 ± 1.1 (4)
6	36.7 ± 0.5** (9)	42.4 ± 1.0* (9)	45.7 ± 0.3 (3)
7	36.0 ± 0.9** (12)	43.6 ± 0.3 (9)	43.3 ± 1.2 (3)
8	33.5 ± 0.7** (9)	43.3 ± 0.3 (9)	43.2 ± 0.6 (4)
9	29.2 ± 1.6** (5)	43.9 ± 0.5 (9)	-
10	16.0 ± 0.0** (2)	43.7 ± 0.3 (9)	-
11	-	-	45.1 ± 0.6 (4)
14	-	-	46.1 ± 0.6 (4)

Each value is the mean ± the SE.

*Significantly different from untreated control mice: $P < 0.005$.

**Significantly different from untreated control mice: $P < 0.0005$.

( ) Indicates number of mice used.

APPENDIX F

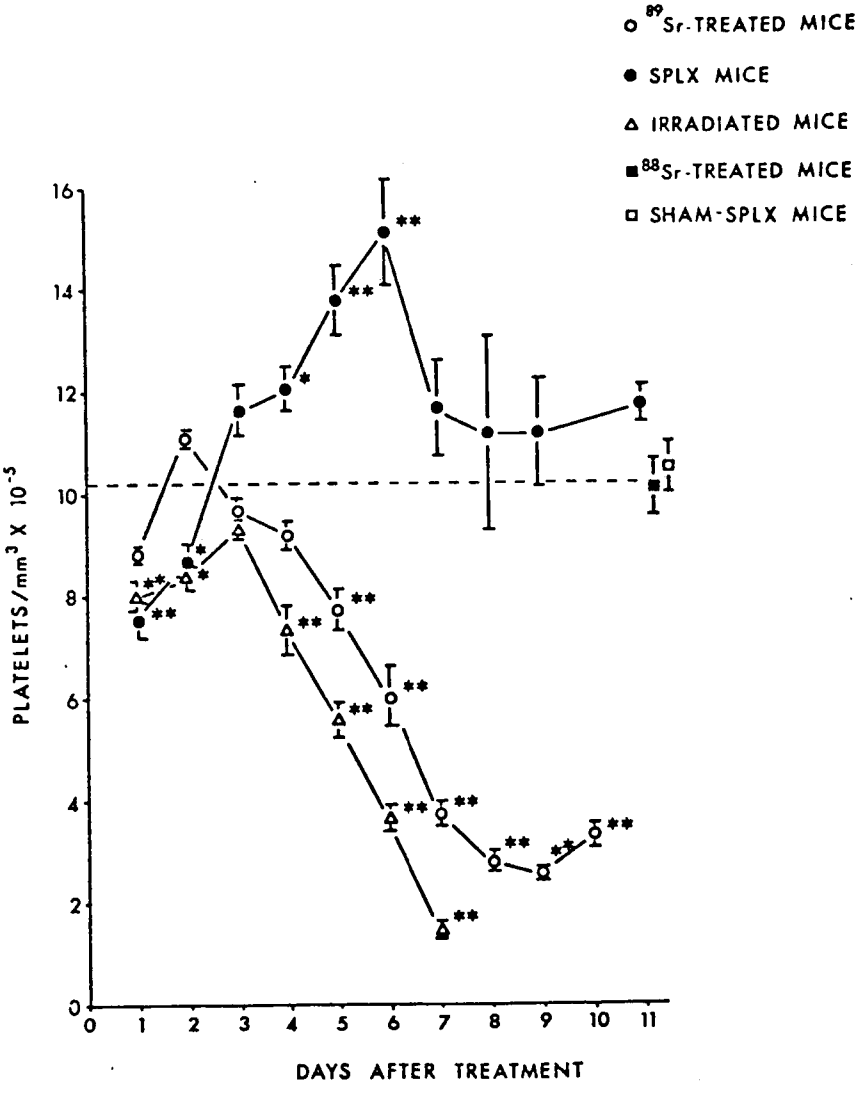

Figure 1. Effect of ^{89}Sr-treatment, splenectomy, and irradiation on platelet counts with time.

Platelet counts of mice are shown following ^{89}Sr injection, splenectomy (Splx), and irradiation (750R). Platelet counts of sham-splenectomized mice and ^{88}Sr-injected mice are represented by single points. The dashed line indicates the mean of 51 untreated mice. Each point is the mean of 3-12 mice. Vertical bars represent the SE. Values were significantly different from control mice: *$P < 0.005$; **$P < 0.0005$.

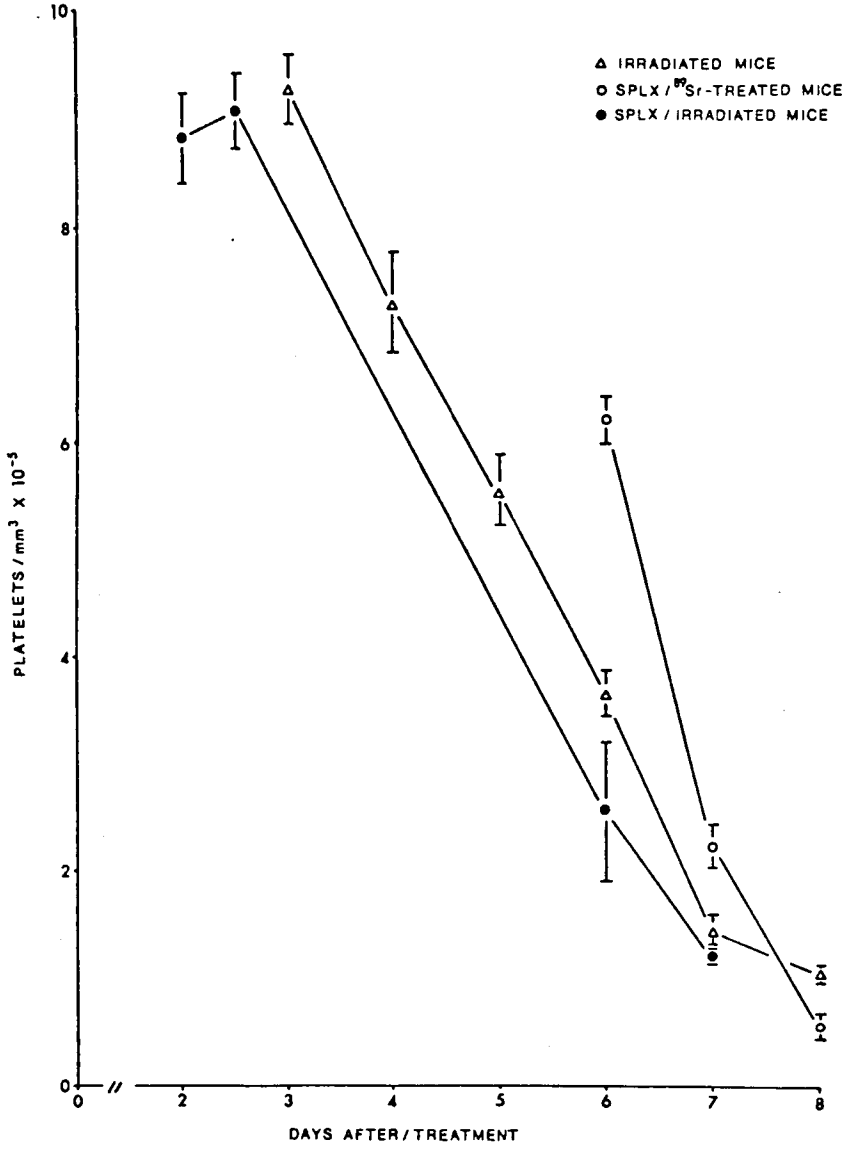

Figure 2. Effect of irradiation, splenectomy (Splx)/^{89}Sr-treatment, and Splx/irradiation on platelet counts with time.

Platelet counts of mice are shown following irradiation (750R), splenectomy and irradiation, and splenectomy and ^{89}Sr-treatment. Each point is the mean of 3-12 mice. Vertical bars represent the SE.

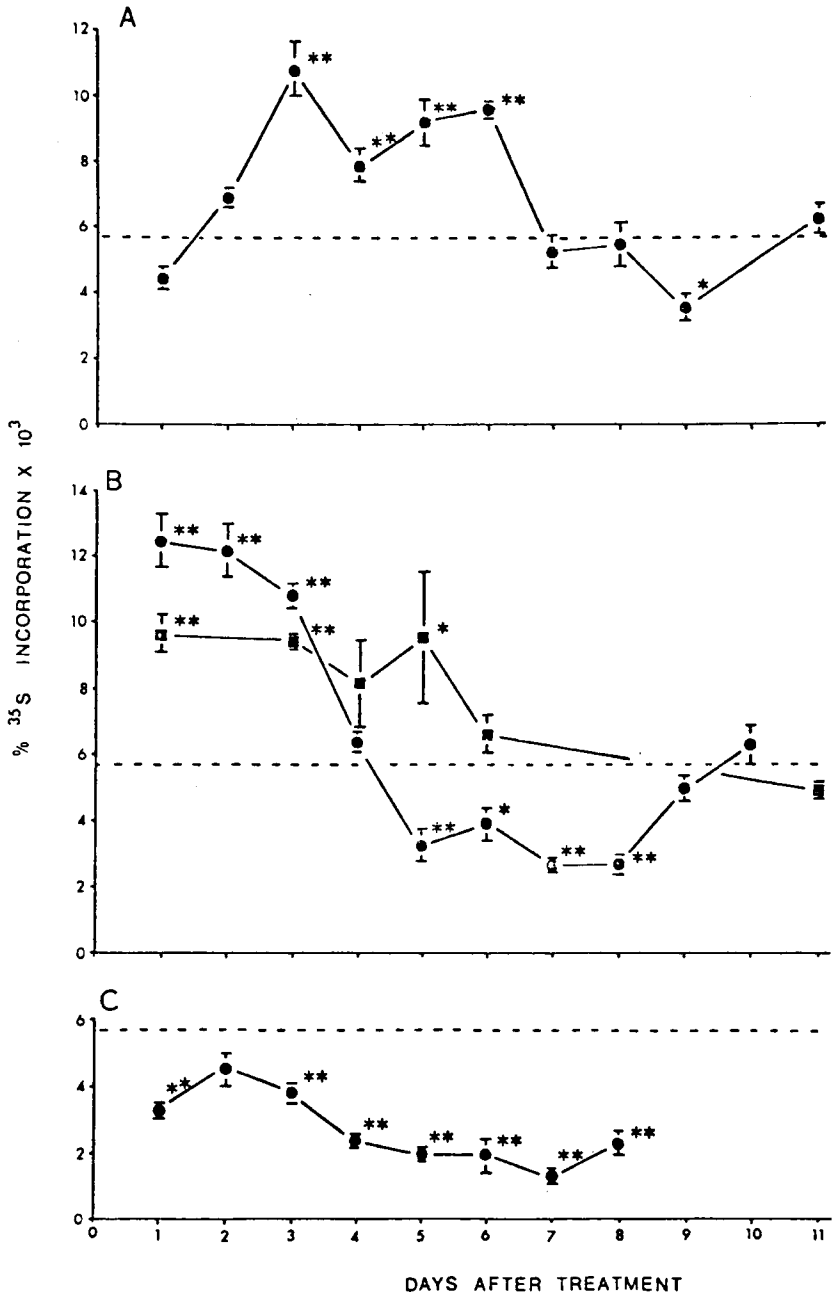

Figure 3. Effect of splenectomy, ^{89}Sr-treatment, and irradiation on percent ^{35}S-incorporation into platelets with time.

Percent ^{35}S-incorporation into platelets twenty-four hours after $Na_2{}^{35}SO_4$ injection is shown following splenectomy (Panel A), ^{89}Sr injection and ^{88}Sr injection (Panel B, ^{89}Sr-treated mice are represented by closed circles, ^{88}Sr by closed squares), and irradiation 750R (Panel C). Each point is the mean of 3-12 mice. The dashed lines represent the mean of 51 untreated mice. Vertical bars represent the SE. Values were significantly different from control mice: *$P < 0.005$; **$P < 0.0005$.

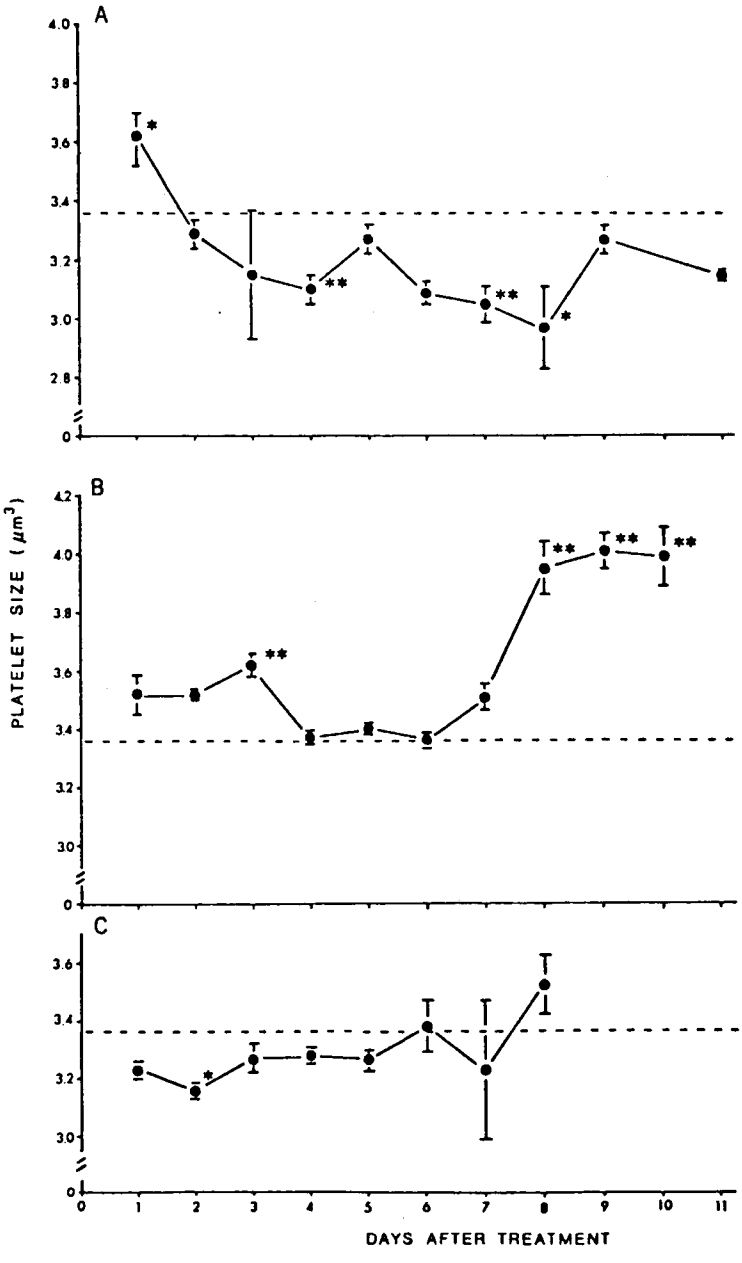

Figure 4. Effect of splenectomy, ^{89}Sr-treatment, and irradiation on platelet sizes with time.

Platelet sizes are shown following splenectomy (Panel A), ^{89}Sr injection (Panel B), and irradiation (750R) (Panel C). Each point is the mean of 3-12 mice. The horizontal dashed lines are the mean of 51 untreated mice. Vertical bars represent the SE. Values were significantly different from control mice: *$P < 0.005$; **$P < 0.0005$.

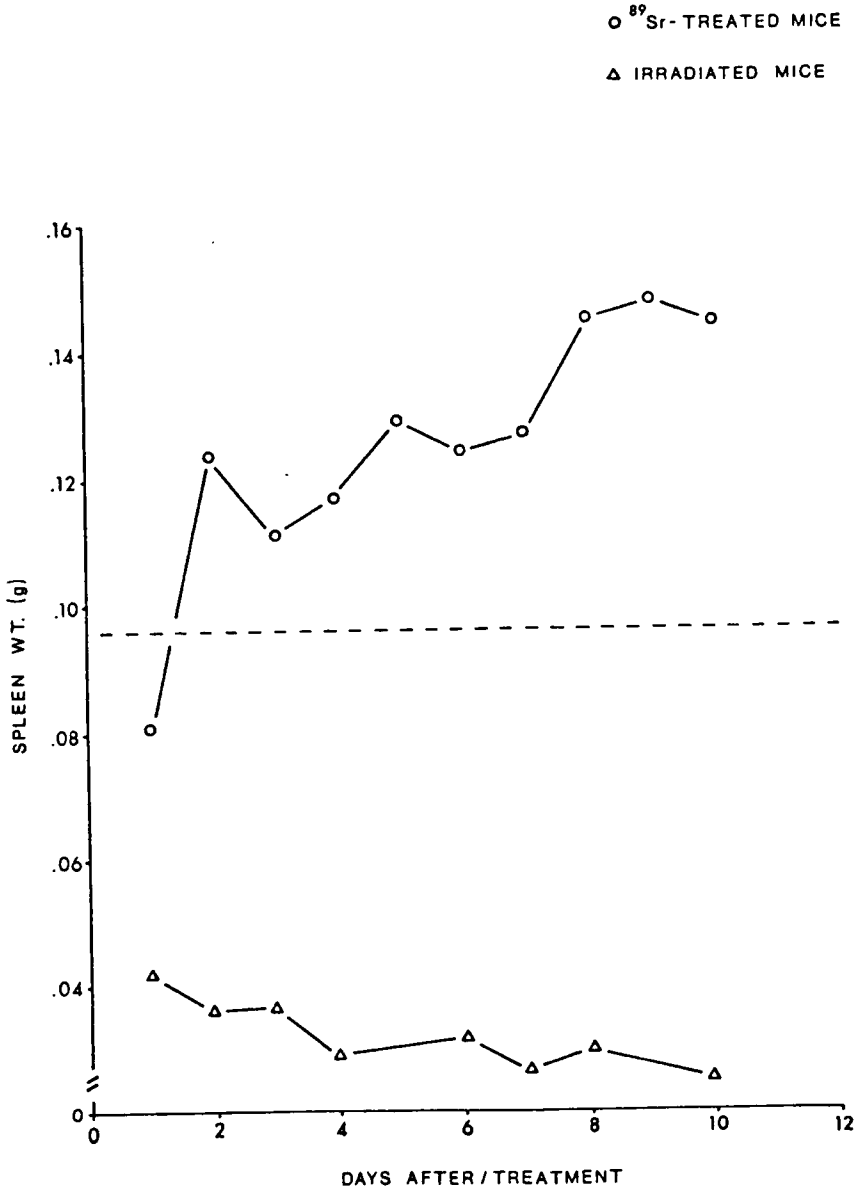

Figure 5. Effect of ^{89}Sr-treatment and irradiation on spleen weights with time.

Spleen weights are shown after ^{89}Sr injection and irradiation (750R). Each value is the mean of two groups of 3 mice each for each day. The dashed line represents the mean spleen weights of 20 untreated mice.

VITA

Stephen James Layendecker was born on July 16, 1957, in Lancaster, Pennsylvania. He attended elementary schools in Landisville, Pennsylvania; Hamilton Square, New Jersey; and Dillsburg, Pennsylvania. He graduated from Northern York High School in June of 1975. The following September he entered Tusculum College, Greeneville, Tennessee. He received a Bachelor of Science degree in Biology-Chemistry in May 1979. In the fall of 1979 he entered the graduate program in Radiation Biology at the University of Tennessee and began study towards a Master's degree.