AFRRI SR66-4
MAY 1966

AFRRI
SCIENTIFIC
REPORT

ERYTHROCYTE STEM CELL KINETICS IN THE POSTRADIATION RAT

S. J. Baum

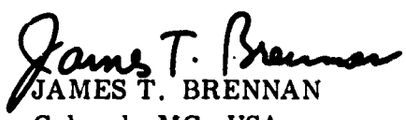
ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

AFRRI SR66-4
May 1966

ERYTHROCYTE STEM CELL KINETICS IN THE POSTRADIATION RAT

S. J. BAUM

S. J. BAUM
Chairman
Experimental Pathology Department



JAMES T. BRENNAN
Colonel, MC, USA
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

ACKNOWLEDGMENT

The skilled technical assistance of J. P. Vagher and D. E. Wyant is greatly appreciated.

ABSTRACT

In rats exposed to 300 R of 250 KVP X-rays, erythropoiesis as measured by ^{59}Fe uptake diminished greatly for 48 hours followed by a rapid recovery approaching near normal values approximately 6 days after radiation. It has been postulated that the rate of recovery was primarily due to accelerated release of noninjured stem cells. The present experiment was designed to test this hypothesis. The polycythemic rat preparation was used since it permits the experimenter to control the release of erythrocyte stem cells. In polycythemic rats observed for 17 days postradiation (300 R of 250 KVP X-rays), stem cell release diminished to 8 percent of the control values during the first 24 hours. This was followed by a rapid recovery from the 2nd to the 5th day. A second decrease was noted from the 6th to the 9th day and a third depression from the 9th to the 12th day. Thereafter, the oscillations diminished indicating a possible return toward the preradiation normal state. An attempt was made to correlate these findings with a kinetic model of erythropoiesis. It was suggested that the initial depression in stem cell release might be due to cellular destruction and inhibitions of cellular release mechanisms. The oscillations of the recovery curve were ascribed to possible rate differences in cellular movements from one precursor compartment to the subsequent one, and to competitive stimulations for progenitor cells from related cellular systems of the hematopoietic system.

TABLE OF CONTENTS

LIST OF FIGURES

I. INTRODUCTION

The injury, subsequent repair and recovery of erythrocyte precursor cells in sublethally irradiated animals have been adequately described.[4,12,5]

Utilizing ^{59}Fe incorporation into newly formed erythrocytes as a measure of erythrocyte production, Baum[2] demonstrated that rats exposed to 300 R (250 KVP) of X-rays show a decrease in erythrocyte production which reached its maximum 2 days after exposure. This was followed by an exponential increase in the rate of appearance of newly formed erythrocytes in the circulatory system until near normal values were measured 6 days postradiation. The acceleration in the rate of red cell production during the recovery period was ascribed to increased stimulation of stem cells for the release of noninjured cells for proliferation. This contention finds support in reports by Erslev[9] and Alpen and Cranmore[1] who observed increases in the number of pronormoblasts within a few hours after animals were phlebotomized. The results obtained by these investigators clearly demonstrated that reduction in total circulatory erythrocyte volume was the stimulus for the sudden increased rate in release of stem cells for proliferation into pronormoblasts. The present experiment was designed to test the hypothesis that postradiation erythropoietic recovery depends on the availability of surviving stem cells. The polycythemic rat preparation was found to be ideal for these studies since it permitted control of the release of stem cells.

II. METHODS

This report comprises four studies employing 358 male albino rats of the Sprague-Dawley strain from the colony of the Simonsen Laboratories, White Bear, Minnesota. At the beginning of each study, the rats were 140 ± 3 days old and weighed from 258 to 308 gm. The animals were housed in individual, clean wire cages with a wire mesh bottom. The food, in the form of baked biscuits obtained from the D and G Laboratory Animal Foods Company, as well as water, were given ad libitum.

The first study was designed to compare maximum radioiron incorporation in normal rats with those of polycythemic animals receiving either erythropoietin-rich plasma or saline. Fifty-six rats were made polycythemic by the intravenous injection of 4 doses of 5 ml of washed homologous erythrocytes in a 7-day period. The mean hematocrit of these animals was raised from 43 to 65 percent and the mean erythrocyte count from 6.5 to 9 million per cu mm. A 2 ml aliquot of erythropoietin-rich plasma was injected into 42 of these rats and an equal volume of saline into the remaining 14 on day 0. These rats were previously divided into 7 groups, one of which was injected with 1 μCi of ^{59}Fe either on the day preceding, or on the same day, or on each of the 5 days subsequent to the erythropoietin administration. In addition to the polycythemic rats, 14 normal rats were injected with the radioactive iron to establish normal maximum uptake values.

The erythropoietin-rich plasma was obtained from rats which were subjected to 500 R of 250 KVP X-rays and concomitant intraperitoneal injection of phenylhydrazine, hydrochloride (50 mg/Kg). These rats became extremely anemic within 3 days

(hematocrit $\leq$ 10), at which time they were exsanguinated and the plasma was separated from the blood. Bioassay with polycythemic rats indicated that 2 ml of this anemic rat plasma elicited an erythropoietic response similar to 6 units of Standard A erythropoietin supplied by the Hematology Study Section, U. S. Public Health Service.

One week after the intravenous injection of ^{59}Fe, 0.25 ml of blood was withdrawn via the jugular vein from each rat of which 0.05 ml was pipetted into a 15 ml stoppered test tube to which 2 ml of distilled water was added. These samples were counted for ^{59}Fe activity in an automatic dual channel well type gamma scintillation counter using a 3 x 3 thallium activated sodium iodide crystal.

Blood volumes were obtained from each rat utilizing the ^{51}Cr methodology developed by Sterling and Gray.[15]

The second experiment was a study designed to measure the rate of stem cell release in the irradiated rat. A group of 42 rats, made polycythemic in the manner described above, were exposed to 300 R of 250 KVP X-rays. Immediately after radiation and on each of the following 6 postradiation days, 6 hypertransfused animals received 2 ml of erythropoietin-rich rat plasma, followed with an intravenous injection of 1 μCi of ^{59}Fe 2 days later. With each irradiated group an identical treatment regime was given to 2 nonirradiated rats. Results from the nonirradiated control group, comprised of 14 rats, were utilized as control values. Blood sampling and testing for radioactivity were conducted as described above.

The third experiment involved 88 polycythemic male rats, 66 of which were exposed to 300 R of 250 KVP X-rays. The treatment of these rats was similar to that in the second experiment except that the period of observation was extended to 10 postradiation days.

The fourth study utilized 108 irradiated (300 R of 250 KVP X-rays) and 36 non-irradiated polycythemic rats, which were treated in the manner described above, and studied for 17 days after exposure.

The radiation source was a General Electric Maxitron X-ray generator with the following radiation factors: 250 KVP, 30 ma, 0.95 mm Cu + 1.2 mm Be filtration (HVL-1.9 mm Cu); and target to subject midline distance 110.5 cm. The midline dose rate was 20 R/min in air.

For the radiation exposure, all rats were placed in Lucite boxes and arranged in the radiation field so that the midline air dose rate was similar for all animals (maximum deviation ± 4%).

The significance of the difference of maximum and minimum points on the oscillating curve was tested by using the ranks test of White.[17]

III. RESULTS

The mean maximum ^{59}Fe incorporation into newly formed erythrocytes of normal rats was 65 percent (Figure 1). In polycythemic rats this decreased to 15 percent. Maximum radioiron uptake increased slightly in these animals if the isotope was injected either the day preceding or immediately after the administration of erythropoietin-rich plasma. It continued to increase until essentially normal values were measured 2 days after the erythropoietin administration. Thereafter, ^{59}Fe incorporation decreased and on the 5th day returned to the baseline values established for polycythemic rats injected with saline. Figure 1 demonstrates, therefore, that the release of stem cells was greatly reduced in the polycythemic rat and that the

administration of erythropoietin returned it to normal levels. Furthermore, the cell population measured by ^{59}Fe uptake 2 days after the erythropoietin injection, was that stimulated by it for release from the stem cells. It is, therefore, a measure of stem cell capabilities at the time of erythropoietin administration.

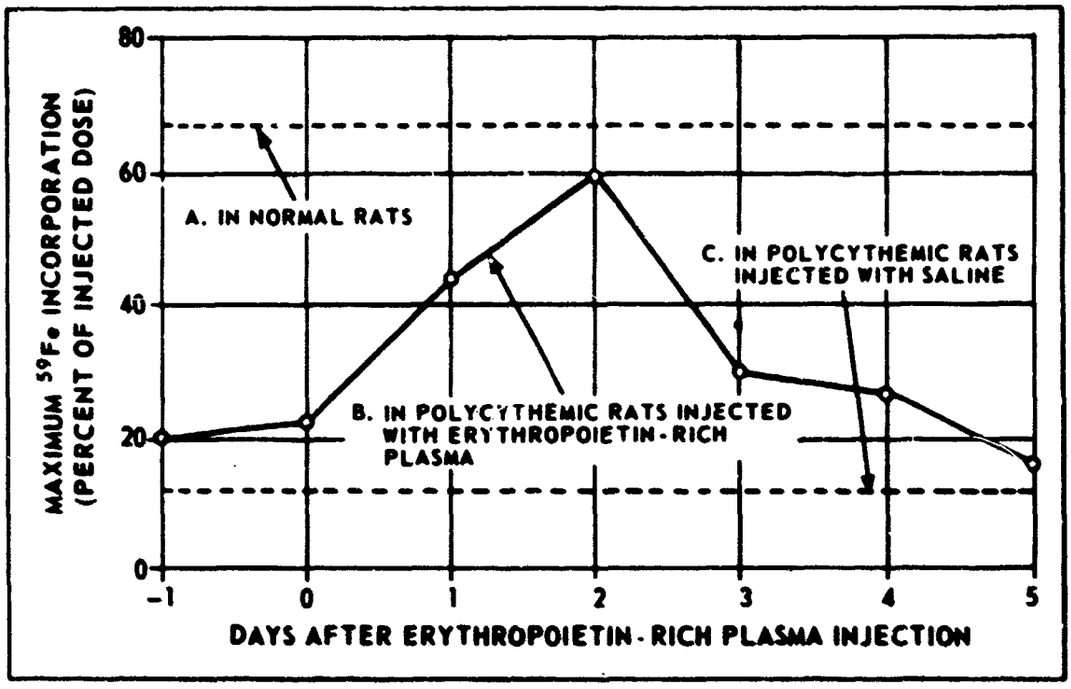


Figure 1. Iron incorporation in normal and polycythemic rats

Polycythemic erythropoietin treated animals exposed to 300 R of X-rays showed a tremendous reduction in radioiron uptake for the first 24-48 hours, with a maximum depression down to 8 percent (Figure 2). Rapid increases are noted from the 2nd to the 4th day postradiation, with no further improvements for the 5th and 6th days. At that time, maximum uptake values were only 70 percent of those observed in non-irradiated erythropoietin treated polycythemic control rats.

When the observation was extended to 10 days postradiation (Figure 3), a second decrease was noted after the 6th day, reaching its lowest point on the 7th day. On the

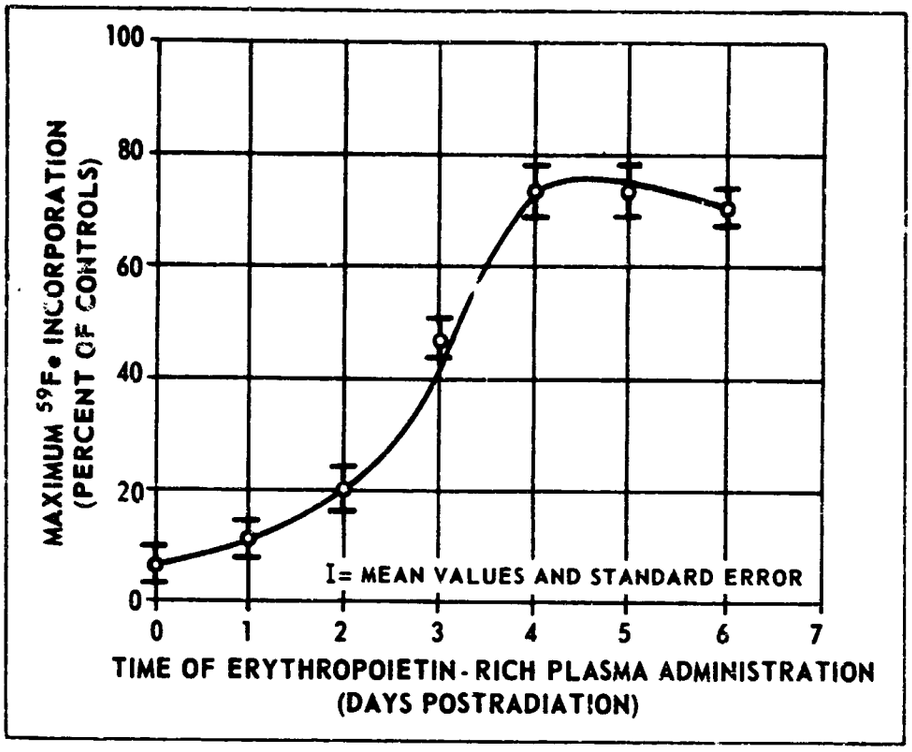


Figure 2. Recovery of erythrocyte stem cells in rats exposed to 300 R of X-rays

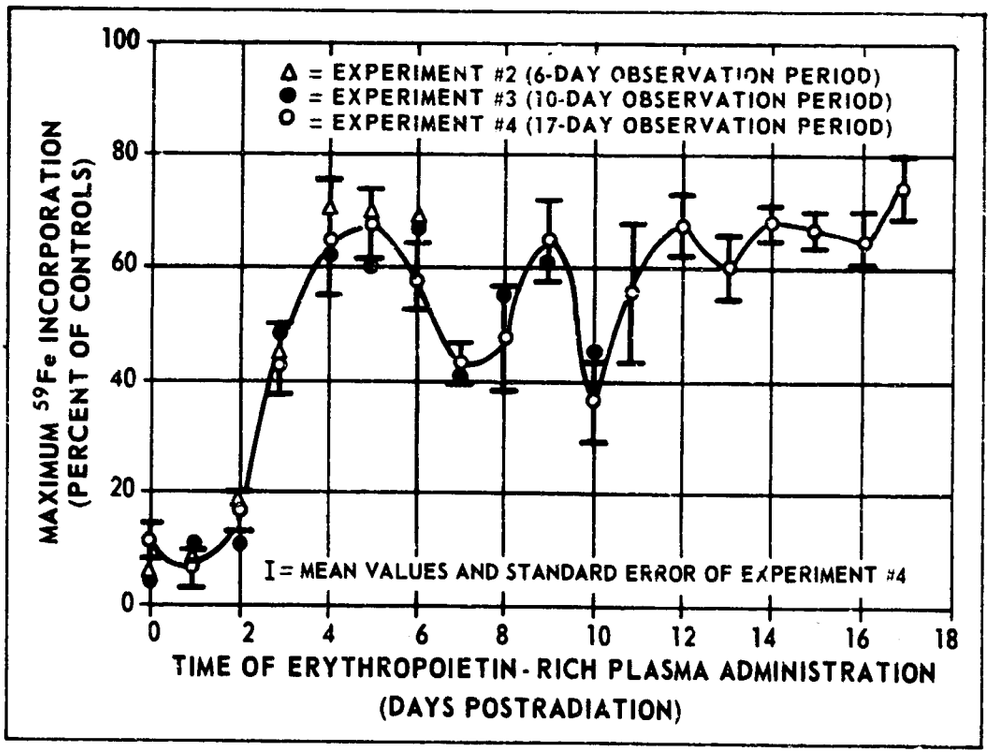


Figure 3. Recovery of erythrocyte stem cells in rats exposed to 300 R of X-rays

9th postradiation day the uptake values had returned only to the level noted on the 4th day and no return to preradiation values was noted.

Final iron incorporation values, obtained on the last day of the 17-day observation period in the fourth experiment, were only 75 percent of those measured in non-irradiated controls. Figure 3 indicates that values obtained from three different groups of rats were quite similar on a given day postradiation. The curve obtained in the last study showed a rapid recovery from the 2nd to the 5th day, a second decrease from the 6th to the 9th day and a third depression from the 9th to the 12th day. Thereafter the oscillations diminished.

The animals utilized for the 10-day observation study were permitted to rest for approximately 3 months. Following this, they were again hypertransfused with washed homologous erythrocytes and injected with erythropoietin-rich plasma. At that time the maximum mean ^{59}Fe uptake was 96 percent of control values. This seemed to indicate that at some time between the 17th and 90th day postradiation, the non-stressed erythropoietic system of the rat regained the preradiation capability to respond to the stimulation of a measured quantity of erythropoietin.

IV. DISCUSSION

The great decrease in radioiron incorporation in polycythemic rats implied a marked reduction of erythropoiesis. Histological observations of Stohlman et al.[16] support this contention. These authors found only a very occasional red cell precursor in the bone marrow of hypertransfused rats. Furthermore, Gurney et al.[10] reported similar findings in mice. This is probably the result of cessation, or at least great

reduction, in the production of endogenous erythropoietin since it may be rectified by its exogenous administration. It is quite apparent that the quantity of administered erythropoietin utilized in the present study elicited maximum stem cell response. Furthermore, as may be seen from the results of the first study, this response reflected the capability of the stem cell compartment to release cells for proliferation at the time of erythropoietin administration.

The results of the present study clearly indicate that immediately after radiation, and for at least the next 24 hours, few stem cells were released. The destruction of a great number of these cells could have been responsible for this condition. However, it would be difficult to postulate a cell renewal within the compartment, which would have to occur at a very rapid rate, in order to explain subsequently observed recovery patterns. Perhaps a more plausible explanation is given below.

Presumably, erythropoietin initiates enzymatic actions which convert multipotential cells into those capable of erythrocyte proliferation. When this has occurred the cells are considered to have been released from the stem cell space. Ionizing radiation may initiate intracellular damage thus impairing the action of these enzyme systems, which in turn, would inhibit the release mechanisms. This, in addition to cellular destruction, could indeed be responsible for the near cessation of cellular release during the 1st day. The occurrence of such intracellular damage and subsequent recovery has been demonstrated in several studies.[7,8]

Since intracellular recovery is accomplished within a number of hours, such cells become available for release upon stimulation by erythropoietin administered beyond the 1st day. If the stem cell space had been reduced to below 10 percent of its normal

size it would be very doubtful that recovery could have progressed as rapidly as it did, or if indeed it could have occurred.

One may postulate that the oscillating recovery curve is the result of the interplay of a number of stimulatory forces initiated by the intricate feedback system attempting to reestablish normal conditions. The radiation injured erythropoietic system will be somewhat limited in this attempt because of reduction in the number of cells capable of release and due to competitive efforts of related systems (i.e., leukopoiesis). Most likely, combinations of these conditions are the cause of the setbacks noted in the recovery curve. For example, the cessation of the rapid recovery on the 5th postradiation day may imply exhaustion of the system but it could also indicate competitive stimulation of multipotential cells for leukopoiesis.[10] Lamerton et al.[14] observed a secondary decrease in ^{59}Fe uptake from the 7th to the 12th day in normal rats exposed to 200 R of X-rays. In view of the results shown in this report, this secondary decrease may have been due to the reactions described above.

It is felt that the recovery curves observed in the present study can best be interpreted by studying their relationship to a hypothetical model of erythropoiesis based on the most recent findings in this field.

Presumably, under normal conditions, cells are released at a constant rate from a primitive progenitor population (PPC's) and due to intracellular changes, become responsive to erythropoietin. This concept appears to be supported by findings of Bruce and McCulloch[6] in mice. However, evidence also has been presented that these erythropoietin responsive cells (ERC's) have multipotential capabilities.[11]

Erythropoietin stimulates the release of the ERC's[9,1] to develop into functional pronormoblasts with the capability for hemoglobin synthesis. Another complex mechanism controls the proliferation of these cells within the precursor compartment, and experimental evidence seems to suggest[13] that they are undergoing three to four cell cycles before they are released as mature erythrocytes into the circulation. Under emergency conditions, cells may be released sooner (i.e., with fewer divisions). This process is called terminal division.[1] On the other hand, it has been suggested that there exists a condition termed ineffective erythropoiesis[16] where cells go through one or two divisions and die. It appears that these two processes are regulatory, possibly under some specific control which might conceivably include erythropoietin.

Erythropoietin produced by the stimulus of hypoxia caused by hemorrhage initiates a rapid release of ERC's into the pronormoblast population.[9,1] The released ERC's are replaced by other cells derived from mitotic division within the compartment and apparently by the transformation of primitive progenitor cells (PPC's) into ERC's. There is evidence that the PPC's are released at a slower rate.[6] Since the cells which are primarily damaged by ionizing radiation are the earlier precursors and stem cells, it is suggested that this condition somehow initiates accelerated erythropoietin production similar and in addition to decreased intracellular pO_2. The recovery curve observed in the present study indicates decreased ERC release for the lst day after radiation, a circumstance which was earlier interpreted as being due to inhibitions of release mechanisms and cellular destruction. This is followed by a rapid rate of recovery which presumably is caused by intracellular recovery of ERC's as well as by their repopulation from the progenitor pool. The accelerated

rate of recovery abruptly stops by the 5th postradiation day and a significant deceleration ensues. This pattern is repeated on the 9th day. The underlying causes for these oscillations are of course complex. However, competitive leukopoiesis has already been suggested as one of them. Another cause may well be the difference in the rate of cellular release from the primitive progenitor pool to the ERC's and from that population to the pronormoblasts. If the transformation of PPC's into ERC's occurs at a slower rate as research by Bruce and McCulloch[6] indicates, and if in response to increased titers of erythropoietin the ERC's differentiate more rapidly into pronormoblasts, their numbers will be decreased. Results obtained in the present studies and depicted in the recovery curve between the 5th and the 12th day seem to support this contention. Eventually the number of primitive progenitor cells released into the ERC compartment in combination with cell renewal within it causes sufficient repopulation to maintain its new steady state.

It is of interest to note that stem cell release for the 17 postradiation days of observation never exceeded 75 percent of values observed in nonirradiated animals. However, previous studies[2] at the same radiation dose level in normal rats revealed nearly complete recovery of erythropoiesis within 6 days. This appears to support the contention that, whereas postradiation recovery is primarily due to accelerated stem cell release, additional adjustments do actually occur within the precursor compartment and may possibly implicate control mechanisms discussed above.

In order to assess the extent of recovery to stem cells, the rat population observed initially for the 10-day postradiation period was again hypertransfused 3 months later and near normal ^{59}Fe uptake values were observed after erythropoietin

administration. This appears to suggest that sufficient recovery occurred so that under nonstressed conditions essentially normal stem cell responses to erythropoietic stimulation may be elicited. However, this does not necessarily imply a complete return to preradiation normalcy of stem cells, since studies in normal[2] as well as polycythemic rats[3] denote a nonrecuperable fraction of the radiation sustained injury.

Finally, it is of interest to point out that the sensitivity of these stem cells to a second radiation exposure would vary in accordance with their physiological state at the time of exposure. The oscillating recovery curve, representing results obtained in the present study, proposes that sensitivity does not necessarily decrease, but rather alternates with time during the early postradiation days.

V. SUMMARY

In rats exposed to 300 R of 250 KVP X-rays, erythropoiesis as measured by ^{59}Fe uptake diminished greatly for 48 hours followed by a rapid recovery approaching near normal values approximately 6 days after radiation. It has been postulated that the rate of recovery was primarily due to accelerated release of noninjured stem cells. The present experiment was designed to test this hypothesis. The polycythemic rat preparation was used since it permits the experimenter to control the release of erythrocyte stem cells.

In polycythemic rats observed for 17 days postradiation (300 R of 250 KVP X-rays), stem cell release diminished to 8 percent of the control values during the first 24 hours. This was followed by a rapid recovery from the 2nd to the 5th day. A second decrease was noted from the 6th to the 9th day and a third depression from the

9th to the 12th day. Thereafter, the oscillations diminished indicating a possible return toward the preradiation normal state.

An attempt was made to correlate these findings with a kinetic model of erythropoiesis. It was suggested that the initial depression in stem cell release might be due to cellular destruction and inhibitions of cellular release mechanisms. The oscillations of the recovery curve were ascribed to possible rate differences in cellular movements from one precursor compartment to the subsequent one, and to competitive stimulations for progenitor cells from related cellular systems of the hematopoietic system.

REFERENCES

1. Alpen, E. L. and Cranmore, D. Observations on the regulation of erythropoiesis and on cellular dynamics by Fe^{59} autoradiography. In: Conference on Fundamental Problems and Technics for the Study of the Kinetics of Cellular Proliferation, pp. 290-300, Salt Lake City, 1959. Stohlman, F., Jr., editor: The Kinetics of Cellular Proliferation, New York, Grune & Stratton, 1959.

2. Baum, S. J. Erythropoietic recovery and residual injury in rats exposed repeatedly to X-rays. Amer. J. Physiol. 200:155-158, 1961.

3. Baum, S. J. Radiation induced residual injury to erythrocyte stem cells. International Congress of Hematology, 10th, p. M:7, Stockholm, 1964.

4. Baxter, C. F., Belcher, E. H., Harriss, E. B. and Lamerton, L. F. Anaemia and erythropoiesis in the irradiated rat: An experimental study with particular reference to techniques involving radioactive iron. Brit. J. Haematol. 1:86-103, 1955.

5. Bloom, W., editor. Histopathology of Irradiation from External and Internal Sources. New York, McGraw-Hill Book Company, 1948.

6. Bruce, W. R. and McCulloch, E. A. The effect of erythropoietic stimulation on the hemopoietic colony-forming cells of mice. Blood 23:216-232, 1964.

7. Elkind, M. M. and Sutton, H. X-ray damage and recovery in mammalian cells in culture. Nature 184:1293-1295, 1959.

8. Elkind, M. M. Repair of X-ray damage in mammalian cells. Japan J. Genet. 40:176-193, 1964.

9. Erslev, A. J. The effect of anemic anoxia on the cellular development of nucleated red cells. Blood 14:386-398, 1959.

10. Gurney, C. W., Lajtha, L. G. and Oliver, R. A method for investigation of stem-cell kinetics. Brit. J. Haematol. 8:461-466, 1962.

11. Gurney, C. W., Hofstra, D. and Mangalik, A. Physiological studies of primitive hemopoietic cells. Argonne Cancer Research Hospital Semiannual Report to the Atomic Energy Commission (ACRH-23), pp. 21-23, 1965.

12. Lajtha, L. G. and Suit, H. D. Uptake of radioactive iron (^{59}Fe) by nucleated red cells <u>in vitro</u>. Brit. J. Haematol. 1:55-61, 1955.

13. Lajtha, L. G. Stem cell kinetics and erythropoietin. In: Conference on Erythropoietin and Regulation of Erythropoiesis, pp. 140-150, San Francisco, 1962. Jacobson, L. O. and Doyle, M., editors: Erythropoiesis, New York, Grune & Stratton, 1962.

14. Lamerton, L. F., Belcher, E. H. and Harriss, E. B. Blood uptake of Fe^{59} in studies of red cell production. In: Conference on Fundamental Problems and Technics for the Study of the Kinetics of Cellular Proliferation, pp. 301-311, Salt Lake City, 1959. Stohlman, F., Jr., editor: The Kinetics of Cellular Proliferation, New York, Grune & Stratton, 1959.

15. Sterling, K. and Gray, S. J. Determination of the circulating red cell volume in man by radioactive chromium. J. Clin. Invest. 29:1614-1619, 1950.

16. Stohlman, F., Jr., Brecher, G. and Moores, R. R. Humoral regulation of erythropoiesis. VIII. The kinetics of red cell production and the effect of erythropoietin. In: Conference on Erythropoietin and Regulation of Erythropoiesis, pp. 162-172, San Francisco, 1962. Jacobson, L. O. and Doyle, M., editors: Erythropoiesis, New York, Grune & Stratton, 1962.

17. White, C. The use of ranks in a test of significance for comparing two treatments. Biometrics 8:33-41, 1952.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) Armed Forces Radiobiology Research Institute Defense Atomic Support Agency Bethesda, Maryland 20014	2a REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b GROUP N/A

3 REPORT TITLE

ERYTHROCYTE STEM CELL KINETICS IN THE POSTRADIATION RAT

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Baum, S. J.

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
May 1966	25	17

8a CONTRACT OR GRANT NO	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO	AFRRI SR66-4
c DB 03.1473	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

10 AVAILABILITY/LIMITATION NOTICES

Distribution of this document is unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Defense Atomic Support Agency Washington, D. C. 20301

13 ABSTRACT

In rats exposed to 300 R of 250 KVP X-rays, erythropoiesis as measured by ^{59}Fe uptake diminished greatly for 48 hours followed by a rapid recovery approaching near normal values approximately 6 days after radiation. It has been postulated that the rate of recovery was primarily due to accelerated release of noninjured stem cells. The present experiment was designed to test this hypothesis. The polycythemic rat preparation was used since it permits the experimenter to control the release of erythrocyte stem cells. In polycythemic rats observed for 17 days postradiation (300 R of 250 KVP X-rays), stem cell release diminished to 8 percent of the control values during the first 24 hours. This was followed by a rapid recovery from the 2nd to the 5th day. A second decrease was noted from the 6th to the 9th day and a third depression from the 9th to the 12th day. Thereafter, the oscillations diminished indicating a possible return toward the preradiation normal state. An attempt was made to correlate these findings with a kinetic model of erythropoiesis. It was suggested that the initial depression in stem cell release might be due to cellular destruction and inhibitions of cellular release mechanisms. The oscillations of the recovery curve were ascribed to possible rate differences in cellular movements from one precursor compartment to the subsequent one, and to competitive stimulations for progenitor cells from related cellular systems of the hematopoietic system.

DD FORM 1 JAN 64 1473

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
erythrocytes radiation effects stem cells ^{59}Fe incorporation erythropoietic recovery postradiation						

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.