Behavior of the Transfused Celullar Elements of Bone Marrow in X-Irradiated Guinea-Pigs*

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I. INTRODUCTION

Blood cells are formed in the hematopoietic tissues and delivered to the circulating blood as they become mature. Under special circumstances, immature cells are also dislodged from the hematopoietic tissues into the general circulation and may settle in the definite areas, often leading to the formation of ectopic foci of blood formation. In this respect, blood cells differ from other tissue elements and resemble tumor cells.

In order to clarify such peculiarities of blood cells, it was first attempted to follow the behavior of the transfused cellular elements of hematopoietic tissues. The chief results of the previous experiments on normal rabbits by *Osogoe* (1950) and *Osogoe* and *Omura* (1950) are as follows: (1) Immediately after intravenous injection, the cellular elements of hematopoietic tissues became blocked in the lung capillaries, and they were then gradually transported by the blood stream to the liver and spleen and accumulated in these organs during the period from the 24th to 72nd hour. (2) Between the cells of the lymphoid and the myeloid series there was a difference in the favorite sites of their accumulation. Namely, chief accumulation of the cells of the lymphoid series occurred in the periportal spaces of the liver and in the perifollicular regions of the spleen, whereas the sites of accumulation of the cells of the myeloid series (erythrocytic and granulocytic series) were the sinusoid of the liver and the red pulp of the spleen.

The second attempt was to dislodge immature cells from the bone marrow into general circulation on a large scale by the intravenous injection of a single large dose of saponin into normal rabbits and to follow the behavior of these cells in foreign organs (*Omura* and *Osogoe*, 1951). It was revealed that the pattern of accumulation of these cells in foreign organs was quite the same as that observed after transfusion of cellular elements of bone marrow from other rabbits.

In both experiments, however, the accumulated immature blood cells were unable

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to continue to proliferate for a long time in foreign situations, although they were very frequently in mitosis up to the 72nd hour. To facilitate the further proliferation of the immature blood cells accumulated in foreign situations, some additional factors seems to be necessary, presumably those which produce insufficiency of the hematopoietic functions.

The purpose of the present work is to follow the behavior of the transfused bone marrow elements in guinea-pigs, whose bone marrow has been severely damaged by total-body exposure to a large dose of X-rays, and to determine whether severe damage of bone marrow really facilitates the proliferation of the transfused marrow cells in the recipients or not.

This work was carried out in the laboratory of Professor *Bunsuke Osogoe* during the period from August 1955 to October 1956. The author wishes to express my appreciation to him for his constant guidance in the course of the research work. A preliminary report covering certain phases of this study was read by *Osogoe* at the VIth International Congress of International Society of Hematology, Boston, August 27–Sept ember 1, 1956. Preliminary data were also discussed at the conference on Dynamics of Proliferating Tissues, Brookhaven National Laboratory, Upton, N. Y., September 5–8, 1956. (The Developmental Biology Conference Series, 1956. Edited by *Dorothy Price*. University of Chicago Press, Chicago, 1958.)

II. MATERIAL AND METHODS

Experiments were carried out on young mature guinea-pigs, which were not inbred in the genetic sense, having an initial body weight varying from 400 to 600 g.

Transfusion of bone marrow cells

Transfusion of bone marrow cells was performed immediatly after the recipient animals had been irradiated with X-rays on the whole body with a dose of 550 r.* This dosis is far greater than the $LD_{50}/30$ days for the guinea-pig, which is estimated to be 150–200 r. The guinea-pig was employed in the present study, because this animal is extremely radiosensitive and because its special leukocytes have distinct pseudoeosinophilic granules like those of the rabbit, so that the appearance of the transfused immature cells of this series can easily be recognized in foreign situations, in which these cells otherwise do not occur.

The method used for preparation of the bone marrow suspension to be transfused was the same as that described previously by *Osogoe* and *Omura* (1950). Marrow used for preparation of cell suspension was taken out from the femur, tibia and humerus of 2–7 adult guinea-pigs and made into a heavy suspension in a physiologic

* The radiation factors were : 160 kvp, 3 ma, 0.5 mm Cu+0.5 mm Al, dose rate in air 12–14 r per minute at a distance of 30 cm.

saline solution. The whole amount of the suspension, 5–6 ml, was injected as a single dose into the subcutaneous vein of the foreleg of the recipient as slowly as possible, after 50,000 units of aqueous penicillin having been added. The total number of the nucleated cells injected into one animal amounted to from 142 to $1{,}021 \times 10.^6$

Another series of irradiated guinea-pigs received comparable amounts of marrow cells that had been severely damaged by heating at 50°C. for 30 minutes in a single intravenous injection ; and still another series of irradiated guinea-pigs were injected intravenously with comparable amounts of marrow cells of adult rabbits also in a single dose. In the former case, the total nucleated cells injected amounted to $2{,}265 - 6{,}930 \times 10^6$; and in the latter, $1{,}380 - 6{,}795 \times 10^6$. Both series of animals served as controls, together with those that had received neither X-irradiation nor transfusion of marrow cells.

At 3, 5 and 10 days after the single injection of the bone marrow suspension after irradiation with X-rays, the animals were killed by a blow on the head followed by decapitation and bled as completely as possible. Immediately thereafter, the following tissues were taken out and fixed in *Zenker*-formol : bone marrow (from femur, sternum and ribs), spleen, liver, lungs, kidneys, adrenals, thymus and mesenteric lymph nodes. The tissues were sectioned in paraffin and the prepared slides were stained with *Mayer*'s acid hemalum and eosin.

Tracer experiments

In order to clarify the pattern of the initial distribution of the injected cells, the suspensions of the bone marrow elements labelled with radioactive phosphorus (P^{32}) were transfused into a series of irradiated guinea-pigs in the same manner as mentioned above. The radioactive marrow cells were obtained from 7 donor guinea-pigs (weighing 300–500 g), each of which had been injected intraperitoneally with P^{32} in a dose of 200 μc per 100 g of body weight 48 hours previously. These cells were washed three times with saline solution before injection in order to remove any freely dialyzable P^{32}. Negligible amounts of radioactivity were found in the wash water after the first wash.

In the tracer experiments, the recipient animals were killed 24 hours after the injection of radioactive marrow cells. The lungs, liver, spleen, bone marrow, mesenteric lymph nodes, kidneys and adrenals were taken out and, after weighing the tissues, they were homogenized with a known volume of saline. 0.5 ml of this homogenate was then plated out on each of a pair of watch glasses coated with a thin film of egg-white and glycerine. They were then dried by keeping at 60°C. for 5 hours in a thermostat and used for measurement of radioactivity with a thin mica end-window type of *Geiger-Müller* tube. The distribution of radioactivity following injection of radioactive marrow cells was compared with the distribution of radioactivity in control animals which had received intravenous administration of radioactivity in the

form of inorganic P^{32}. All activity measurements were corrected back to the day of administration. The total weight of the bone marrow was taken as 2 per cent of the body weight.

III. DISTRIBUTION OF THE TRANSFUSED BONE MARROW CELLS LABELLED WITH RADIOACTIVE PHOSPHORUS

In the first series of experiments, the distribution of the transfused bone marrow cells labelled with radioactive phosphorus (P^{32}) in various organs of irradiated guinea-pigs was investigated. The experimental conditions in individual cases were as follows:

Experiment 1: A male guinea-pig weighing 466 g received a single intravenous injection of bone marrow cell suspension after 550 r of total-body X-irradiation. The total number of nucleated cells was 618×10^6. Marrow used for preparation of cell suspension was taken from 7 donor guinea-pigs (weighing 300–500 g), each of which had been injected intraperitoneally with inorganic radioactive phosphorus (P^{32}) in a dose of 200 μc per 100 g of body weight 48 hours previously. The total amount of radioactivity of the injected suspension was estimated to be 0.174 μc. A dilute solution of inorganic P^{32} was injected intravenously in a dose of 0.174 μc into another male guinea-pig weighing 447 g. The latter animal was used as control for the former. Both animals were killed 24 hours after injection.

Experiment 2: A male guinea-pig weighing 478 g was injected intravenously with a marrow cell suspension containing 618×10^6 nucleated cells in a single dose. Marrow was taken from 7 guinea-pigs (weighing 300–500 g) that had received intraperitoneally inorganic P^{32} in the same dosis and in the same manner as in Experiment 1. The total amount of radioactivity of the injected suspension was found to be 0.174 μc. As control, another male guinea-pig weighing 420 g was injected intravenously with a dilute solution of inorganic P^{32} in a dose of 0.174 μc. Both animals were killed 24 hours after injection.

Experiment 3: A male guinea-pig weighing 430 g received a single intravenous injection of the marrow cell suspension containing $1{,}021 \times 10^6$ nucleated cells. Marrow was taken from 7 donor guinea-pigs (weighing 300–500 g) that had received inorganic P^{32} in the same dosis and in the same manner as in the foregoing experiments. The total amount of radioactivity of the injected suspension was estimated to be 0.322 μc. As control, another male guinea-pig weighing 466 g was injected intravenously with a dilute solution of inorganic P^{32} in a dose of 0.322 μc. Both animals were killed 24 hours after injection.

The results of measurements of the amounts of radioactivity in various organs 24 hours after transfusion or irradiation are summarized in Table 1. In this table the relative radioactivity of an organ was expressed as percentage activity to the total activity of all the organs examined.

Table 1. Distribution of radioactivity 24 hours after injection of radioactive marrow cells or inorganic P^{32} into X-irradiated guinea-pigs.

Organ	Injected matter	Experiment 1			Experiment 2			Experiment 3		
		c p m* per g	c p m per organ	Rel.** act. (%)	c p m per g	c p m per organ	Rel. act. (%)	c p m per g	c p m per organ	Rel. act. (%)
Lungs	Marrow cells	2,000	6,880	3.1	1,669	7,344	4.1	1,449	5,579	4.8
	Inorg. P^{32}	181	669	6.8	731	3,034	14.3	931	4,748	8.1
Liver	Marrow cells	2,647	43,918	20.2	2,242	42,374	23.8	3,448	72,925	63.0
	Inorg. P^{32}	184	3,809	39.0	686	11,758	55.6	1,101	27,657	46.3
Spleen	Marrow cells	23,067	8,536	3.9	17,203	9,462	5.3	8,868	4,642	4.0
	Inorg. P^{32}	307	232	2.3	543	320	1.5	1,009	555	0.9
Bone marrow	Marrow cells	15,846	147,843	68.1	11,571	110,619	61.8	3,069	26,393	22.5
	Inorg. P^{32}	328	2,932	30.0	445	4,138	19.6	1,626	19,870	35.6
Mesenteric lymph nodes	Marrow cells	4,520	904	0.4	4,915	1,475	0.8	2,193	768	0.7
	Inorg. P^{32}	215	140	1.5	765	207	0.9	503	186	0.3
Kidneys	Marrow cells	1,672	8,195	3.7	1,542	6,939	3.8	1,958	6,168	5.2
	Inorg. P^{32}	413	1,859	19.1	364	1,452	6.9	786	4,849	8.3
Adrenals	Marrow cells	2,357	943	0.4	1,809	811	0.4	1,624	1,056	0.9
	Inorg. P^{32}	340	129	1.3	457	247	1.1	951	523	0.9

* Counts per minute.

** Relative activity: expressed as percentage activity to the total activity of all the organs examined.

In this series of experiments, the animals that had received radioactive bone marrow cells were autopsied 24 hours after transfusion, because earlier studies (*Osogoe*, 1950; *Osogoe* and *Omura*, 1956) have demonstrated that the injected cells first become blocked in the capillaries of the lung and it is from the 24th hour on that they accumulate in other organs such as liver, spleen, bone marrow and so forth. In their transfusion experiments of lymphocytes labelled with P^{32}, *Monden* et al. (1956) further found that the pattern of distribution of radioactivity in various organs at 48 and 72 hours after transfusion did not show any essential difference from that observed at 24 hours.

Among the data shown in Table 1, the most striking one is that in the animals injected with radioactive marrow cells the radioactivity per gram of fresh tissue was far greater in the spleen and bone marrow, the former in particular, than in other organs; whilst this was not the case in the control animals injected with comparable

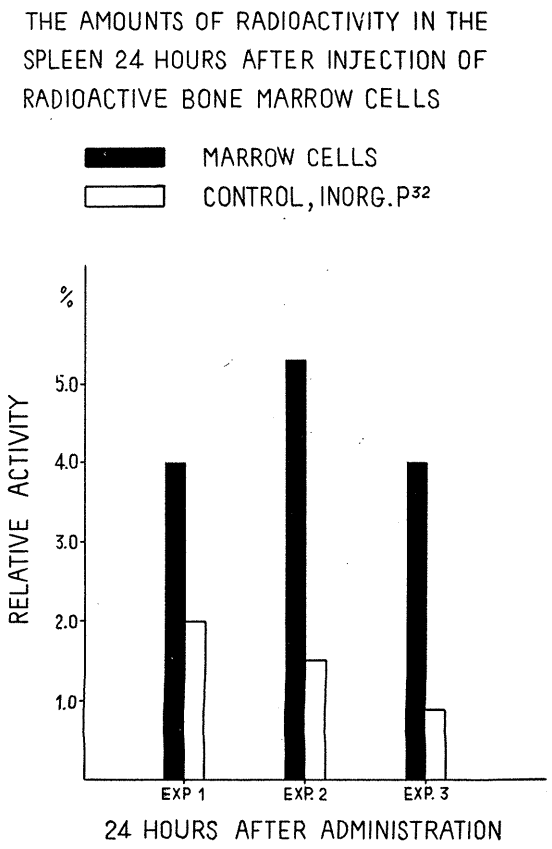

Fig. 1. The amounts of radioactivity in the spleen 24 hours after injection of radioactive bone marrow cells. The heights of the columns indicate the relative activity per organ (lungs + liver + spleen + bone marrow + mesenteric lymph nodes + kidneys + adrenals = 100).

amounts of inorganic P^{32}. The results indicate that the injected radioactive marrow cells had accumulated in these organs to much greater extent than in other organs. In addition, the following findings are also noteworthy : (1) The relative activity of the spleen as a whole was definitely higher after transfusion of radioactive marrow cells than after injection of inorganic P^{32} (Fig. 1). (2) In two of three cases of marrow cell transfusion the relative radioactivity of the bone marrow as a whole was greater than 60 per cent (Fig. 2), whereas the corresponding value of the liver was as low as about 20 per cent. In the case of transfusion of P^{32}-labelled lymphocytes into splenectomized rats, the relative activity of the bone marrow did not exceed the value of the liver (*Monden* et al., 1956).

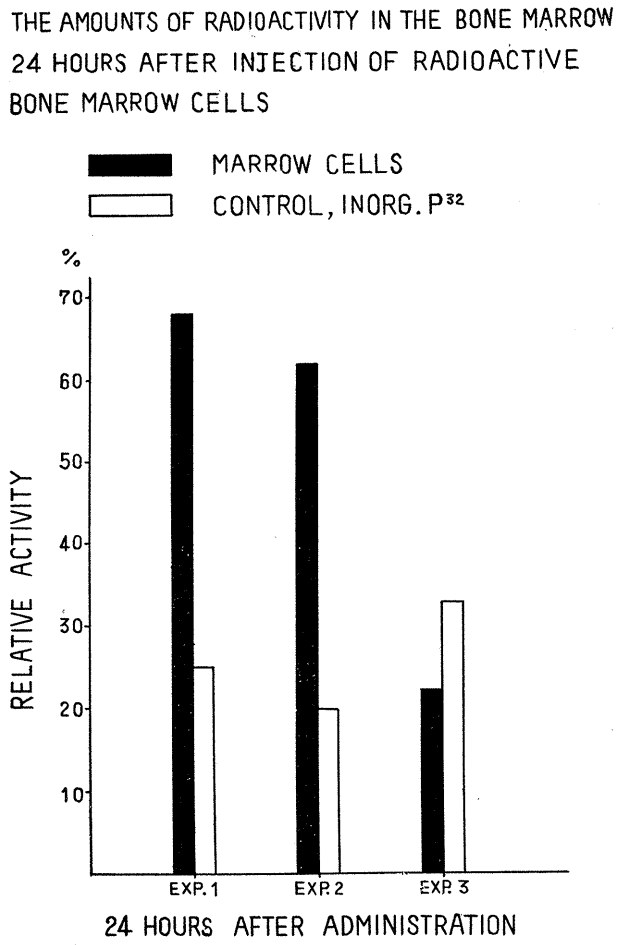

Fig. 2. The amounts of radioactivity in the bone marrow 24 hours after injection of radioactive bone marrow cells. The heights of the columns indicate the relative activity per organ (lungs + liver + spleen + bone marrow + mesenteric lymph nodes + kidneys + adrenals = 100).

IV. MYELOPOIESIS IN THE SPLEEN AND REGENERATION OF BONE MARROW AFTER TRANSFUSION OF INTACT, DAMAGED AND HETEROLOGOUS BONE MARROW CELLS

A. Transfusion of intact bone marrow cells

In this group of experiments, guinea-pigs weighing 400–600 g that had been irradiated with X-rays on the whole body with a dose of 550 r, were injected intravenously with a saline suspension of bone marrow prepared from 3–5 normal guinea-pigs weighing 300–500 g. The injection was made into the subcutaneous vein of the foreleg of the recipient immediately after the animals had received X-irradiation. The animals of the control group received X-irradiation without transfusion of bone marrow cells.

3 days after transfusion

Three guinea-pig weighing around 450 g received a single intravenous injection of marrow cell suspension containing approximately 400×10^6 nucleated cells each immediately after the exposure to 550r of total-body X-irradiation. Despite the injection of large amount of marrow cells, there was no indication either of active proliferation of myeloid cells (myelopoiesis) in the spleen or of regeneration of bone marrow. In the spleen, the periarterial lymphoid tissue disappeared almost completely and the cellularity of the red pulp was greatly reduced. The bone marrow was almost completely depleted of proliferating cells with marked dilatation and congestion of the sinusoids. In the liver, erythroblasts occurred in small numbers scattered in the sinusoids but without formation of cell nests. In the lung, megakaryocytes were occasionally blocked in the lung capillaries. No marrow cells were found either in the lymph nodes, kidneys, or adrenals.

In the control group of 3 guinea-pigs irradiated with X-rays without marrow cell transfusion, bone marrow elements did not occur in any tissue other than the bone marrow.

5 days after transfusion

Three guinea-pigs weighing around 460 g received a single intravenous injection of marrow cell suspension containing $300–500 \times 10^6$ nucleated cells immediately after exposure to 550 r of total-body X-irradiation. On the fifth day after injection, active proliferation of myeloid cells, erythroblast in particular, and regeneration of lymphoid tissue began to take place in the spleen. In one of three cases relatively large foci of erythroblasts were found in the red pulp, both intravascularly in the venous sinuses and extravascularly in the *Billroth*'s cords. In the bone marrow, a slight degree of regeneration was recognized in two of three cases. Beginning regeneration of bone marrow was characterized by predominance of proliferating cells of the erythrocytic series. This was also confirmed by the examination of bone marrow, in which the percentage of erythroblasts was found to be 23 per cent on the average,

whilst the corresponding figure in the control group was less than 10 per cent.

Of particular interest in this connection is the finding that in two of three cases small foci of erythroblasts were found in the sinusoid of the liver. Marrow cells did not occur in other organs, such as the lymph nodes, kidneys, adrenals and lungs. Generally, after transfusion of bone marrow cells into guinea-pigs, it was very difficult to find the injected marrow cells in the liver, spleen and other organs during the period from 24 to 72 hours after injection. In contrast, as reported previously by *Osogoe* and *Omura* (1950), there occurred numerous marrow cells in these organs after transfusion of bone marrow cells into rabbits.

In the control group of 3 guinea-pigs irradiated with X-rays without marrow cell transfusion, there was no indication either of myelopoiesis in the spleen or of regeneration of bone marrow. Likewise, no marrow cells were found in any other organs. As already mentioned, the percentage of erythroblasts in marrow imprints was found to be less than 10 per cent in the control group, whereas the corresponding value in the experimental group that had received marrow cell transfusion was 23 per cent on the average.

10 days after transfusion

By the tenth day, the activity of myelopoiesis in the spleen was greatly intensified and extensive regeneration of bone marrow was often observed. The experimental conditions and histological findings in the spleen and bone marrow, in both the experimental and control groups, are summarized in Table 2.

As seen in this table, extensive proliferation of myeloid cells in the spleen, erythroblasts in particular, was observed in 2 of 6 cases. In such instances, myeloid cell

Table 2. Myelopoiesis in the spleen and regeneration of bone marrow of irradiated rats, with or without transfusion of bone marrow cells, 10 days after irradiation.

Rat no. and sex	Body weight before irradiation (g)	Body weight after irradiation (g)	Spleen weight (g)	No. of injected nucleated cells $\times 10^6$	Myelopoiesis in the spleen	Regeneration of bone marrow
Experimental group: with transfusion of bone marrow cells						
No. 1, ♀	530	500	1.45	302	Extensive	Extensive
No. 2, ♀	470	480	1.05	142	Extensive	Extensive
No. 3, ♂	530	420	0.45	425	Moderate degree	Extensive
No. 4, ♂	420	402	0.35	672	Slight degree	Moderate degree
No. 5, ♂	410	358	0.33	672	Slight degree	Moderate degree
No. 6, ♀	430	390	0.90	715	Moderate degree	Extensive
Control group: without transfusion of bone marrow cells						
No. 1, ♂	490	490	0.27	None	None	None
No. 2, ♂	490	420	0.27	None	None	None
No. 3, ♂	500	515	0.27	None	None	None
No. 4, ♀*	420	490	0.30	None	None	None
No. 5, ♂*	480	460	0.23	None	None	None
No. 6, ♀*	420	470	0.25	None	None	None

* Killed 7 days after irradiation.

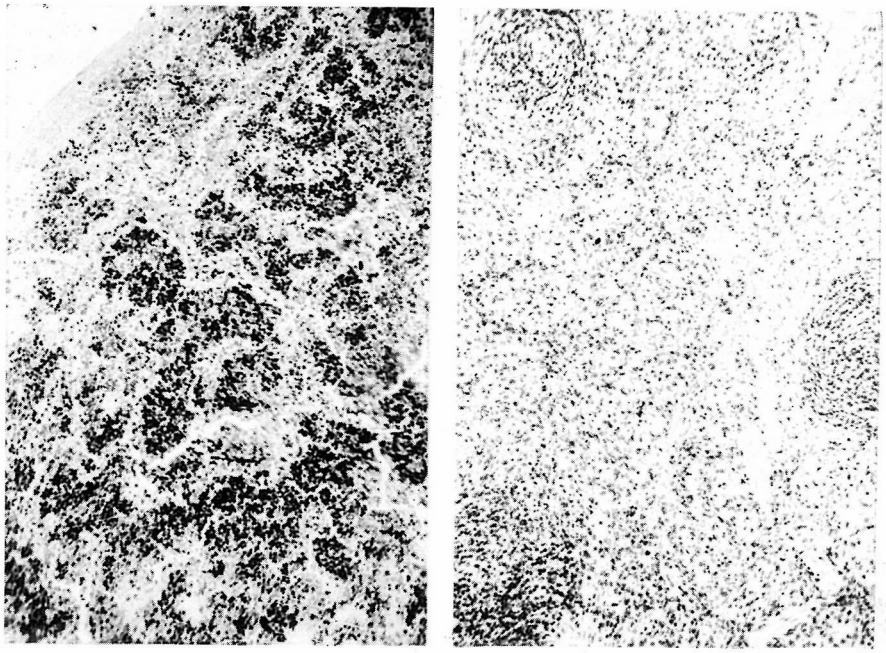

Fig. 3. *Left*: Section of the spleen from a guinea-pig that received a whole-body X-irradiation with a dose of 550 r, showing extensive proliferation of myeloid cells, erythroblasts in particular, in the red pulp, 10 days after marrow cell transfusion. *Right*: Section of the spleen from a control guinea-pig that received a whole-body X-irradiation with a dose of 550 r but without marrow cell transfusion, 10 days after irradiation. The spleen is greatly depleted of proliferating cells.

proliferation took place in the red pulp, and for the most part extravascularly in the *Billroth*'s cords (Fig. 3). A few megakaryocytes were also found therein. Besides, a slight degree of regeneration of the periarterial lymphoid tissue was observed. In the control group without marrow cell transfusion, neither proliferation of myeloid cells nor regeneration of the periarterial lymphoid tissue was observed in the spleen (Table 2).

It is of interest to note here that the spleens with extensive myelopoiesis were much greater in weight than those with lesser degree of myelopoiesis. It seems that extensive proliferation of myeloid cells in the spleen would have resulted in a marked enlargement of this organs (cf. Table 2).

Regeneration of bone marrow at 10 days after transfusion was more pronounced. In 4 of 6 cases, extensive recovery of marrow parenchyma was observed, as illustrated in Fig. 4, and it was also found that proliferating cells of the erythrocytic series far predominated over those of the granulocytic series. An examination of marrow imprints confirmed this, as will be mentioned below. In the control group without marrow cell transfusion, there was no indication of marrow regeneration until the

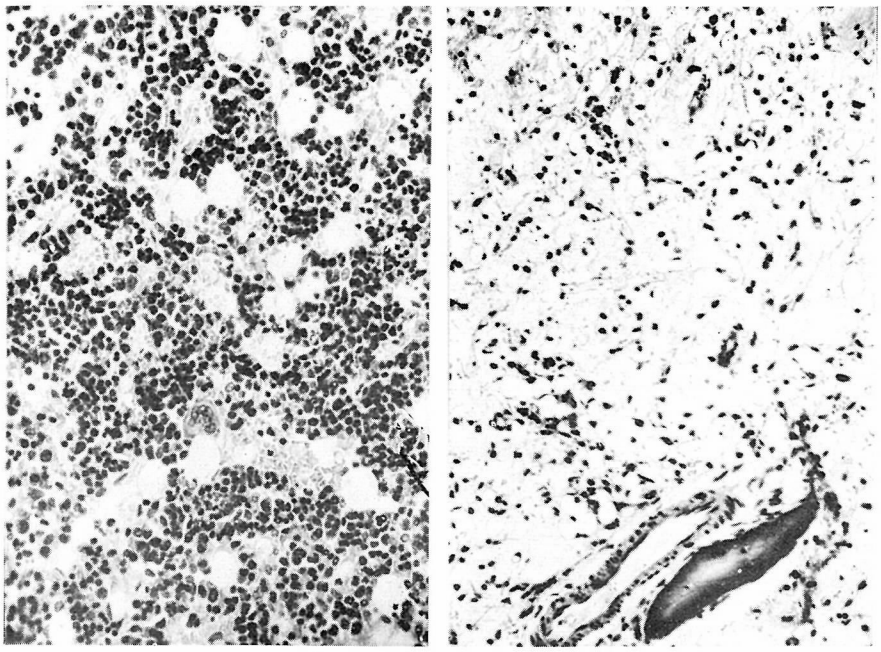

Fig. 4. *Left*: The section of the femoral bone marrow from the same animal as shown in Fig. 3 left, showing almost complete repopulation of hematopoietic tissue, 10 days after marrow cell transfusion. *Right*: Section of the femoral bone marrow from the same control animal as shown in Fig. 3 right, 10 days after irradiation. The marrow is almost completely devoid of hematopoietic cells.

10th day after irradiation (Table 2).

It should be added here that transfusion of bone marrow cells did not elevated the numbers of either leukocytes or platelets until the 10th day (cf. Figs. 5 and 6).

Examination of the spleen and marrow imprints appears to provide more detailed quantitative and qualitative information concerning the hematopoietic recovery, although the pattern of the recovery process was the same as that disclosed by histological examination. It was found that the spleen of normal adult guinea-pigs often participates, though to a very slight degree, in both erythropoiesis and granulopoiesis (cf. Table 3). This, together with lymphocytopoiesis, was greatly reduced 10 days after total-body exposure to 550r of X-rays (cf. Table 4). Marrow cell transfusionin to irradiated animals, on the other hand, resulted in a marked increase in the percentage of erythroblasts. As seen in Table 5, the percentage of erythroblasts in the spleen imprints was greater than 30 per cent in 2 of 6 cases, and in one case as high as 58 per cent at 10 days after the marrow cell transfusion. Although simultaneously a slight elevation was observed in the percentage of myelocytes, the percentage of lymphocytes in the spleen imprints did not show any noticeable increase.

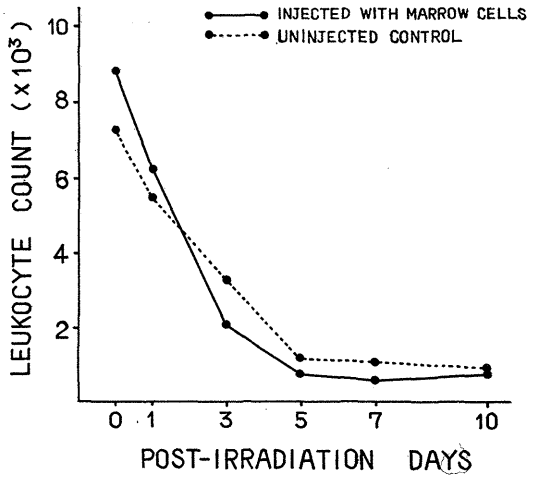

Fig. 5. Changes in the leukocyte count after transfusion of bone marrow cells into X-irradiated guinea-pigs.

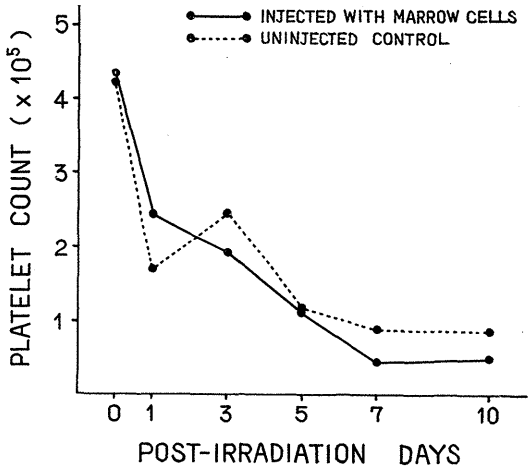

Fig. 6. Changes in the platelet count after transfusion of bone marrow cells into X-irradiated guinea-pigs.

In the bone marrow imprints, the recovery of erythropoiesis 10 days after marrow cell transfusion was found to be much more pronounced than in the spleen imprints. At 10 days after irradiation the percentage of erythroblasts in the marrow imprints was greatly reduced, form a normal average of 40.16 per cent to 9.53 per cent. In contrast, the corresponding figure after marrow cell transfusion was as high as 43.43 per cent on the average (cf. Tables 6–8).

Table 3. Spleen differential cell count in normal guinea-pigs.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4	No. 5	Mean
1. Proerythroblasts	0	0	0	0	0	0
2. Macroblasts	0	0	0	0	0	0
3. Normoblasts	0	0. 1	0. 1	0	0	0. 04
4. Myeloblasts	0	0	0	0	0	0
5. Progranulocytes	0	0	0	0	0	0
6. N. Myelocytes	1. 7	2. 3	0. 5	0. 6	0. 4	1. 10
7. Immature Mc.	0	0	0	0	0	0
8. Half-mature Mc.	0. 1	0. 1	0	0	0	0. 04
9. Mature Mc.	1. 6	2. 2	0. 5	0. 6	0. 4	1. 06
10. N. Metamyelocytes	0. 6	0. 3	0. 2	0. 1	0	0. 24
11. N. Band Cells	0. 1	0	0. 2	0. 1	0. 2	0. 01
12. N. Segmented Cells	7. 3	3. 1	9. 4	4. 8	15. 6	8. 04
13. Eosinophils	0. 9	0	4. 0	0. 1	5. 2	2. 04
14. Basophils	0	0	0. 2	0	0. 2	0. 08
15. Monocytes	0	0	0. 2	0. 1	0. 1	0. 08
16. Lymphoblasts	0. 3	0	0	0. 2	0	0. 10
17. Lymphocytes	60. 0	67. 3	60. 2	70. 3	55. 1	62. 58
18. Megakaryocytes	0	0	0	0	0	0
19. Plasmocytes	0	0	0	0	0	0
20. Reticulo-endothelial Cells	19. 3	24. 4	23. 9	23. 1	21. 8	22. 50
21. Unclassified Cells*	0	0	0	0	0	0
22. Disintegrated Cells	9. 8	2. 5	1. 1	0. 6	1. 4	3. 08

* Small lymphocyte-like cells.

Table 4. Spleen differential cell count in guinea-pigs that received total body X-irradiation with a dose of 550 r. 10 or 7 days after irradiation.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4*	No. 5*	No. 6*	Mean
1. Proerythroblasts	0	0	0. 1	0	0	0	0. 02
2. Macroblasts	0	0	0	0	0	0	0
3. Normoblasts	0	0	0. 3	0. 2	0. 6	0. 4	0. 25
4. Myeloblasts	0	0	0	0	0	0	0
5. Progranulocytes	0	0	0	0	0	0	0
6. N. Myelocytes	0. 3	0. 8	0. 1	0. 4	0. 8	0. 9	0. 55
7. Immature Mc.	0	0	0	0	0	0	0
8. Half-mature Mc.	0	0. 1	0	0	0	0	0. 02
9. Mature Mc.	0. 3	0. 7	0. 1	0. 4	0. 8	0. 9	0. 53
10. N. Metamyelocytes	0	0. 1	0. 2	0. 1	0. 4	0	0. 13
11. N. Band Cells	0. 1	0	0. 2	0	0	0. 1	0. 07
12. N. Segmented Cells	3. 4	0. 5	1. 1	0. 8	0. 9	0. 6	1. 22
13. Eosinophils	0. 7	0	0	0	0. 2	0	0. 15
14. Basophils	0	0	0	0	0	0	0
1c. Monocytes	0	0	0	0. 5	0	0	0. 08
16. Lymphoblasts	0	0	0	0	0	0	0
17. Lymphocytes	3. 6	9. 0	1. 8	18. 8	26. 0	30. 4	14. 93
18. Megakaryocytes	0	0	0	0	0	0	0
19. Plasmocytes	0	0	0	0	0	0	0
20. Reticulo-endothelial Cells	34. 6	39. 9	90. 2	40. 5	33. 9	54. 5	48. 93
21. Unclassified Cells**	0. 7	0. 1	0	0. 2	0. 2	0	0. 2
22. Disintegrated Cells	56. 6	49. 6	5. 1	38. 5	37. 0	12. 1	33. 15

* 7 days after irradaiation.
** Small lymphocyte-like cells.

Table 5. Spleen differential cell count in guinea-pigs that received a transfusion of bone marrow cells immediately after total body X-irradiation with a dose of 550r. 10 days after transfusion.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Mean
1. Proeythroblasts	0	0.1	0	0	0	0	0.02
2. Macroblasts	3.7	1.1	0	0.1	0	0.3	0.87
3. Normoblasts	54.3	30.1	1.7	0.7	0.4	3.6	15.15
4. Myeloblasts	0	0	0	0	0	0	0
5. Progranulocytes	0	0	0	0	0	0	0
6. N. Myelocytes	3.2	3.1	1.8	0.8	0.3	2.9	2.02
7. Immature Mc.	0	0	0	0	0	0	0
8. Half-mature Mc.	0.4	0.1	0	0	0	0.3	0.13
9. Mature Mc.	2.8	3.0	1.8	0.8	0.3	2.6	1.88
10. N. Metamyelocytes	0.7	0.2	0.4	0.1	0.1	0.5	0.33
11. N. Band Cells	0	0	0.7	0.8	0	0.3	0.30
12. N. Segmented Cells	0.6	0.6	4.9	17.3	7.0	5.3	5.95
13. Eosinophils	0	0	0.3	0.1	0.1	0	0.08
14. Basophils	0	0.2	0.1	0	0	0	0.05
15. Monocytes	0.1	0	0	0	0	0	0.02
16. Lymphoblasts	0	0	0	0	0	0	0
17. Lymphocytes	0.8	0.2	2.9	30.8	2.0	19.5	9.37
18. Megakaryocytes	0	0	0	0	0	0	0
19. Plasmocytes	0	0	0	0	0	0	0
20. Reticulo-endothelial Cells	30.6	63.3	79.9	41.3	47.0	62.5	54.10
21. Unclassified Cells*	0	0	0	0	0	0	0
22. Disintegrated Cells	6.0	1.1	7.3	8.0	43.1	5.1	11.77

* Small lymphocyte-like cells.

Table 6. Bone marrow differential cell count in normal guinea-pigs.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4	No. 5	Mean
1. Proerythroblasts	0.1	0.2	0	0	0	0.06
2. Macroblasts	1.4	0.8	2.7	14.7	1.4	4.20
3. Normoblasts	39.9	41.4	41.0	33.0	45.5	40.16
4. Myeloblasts	0.1	0.3	0.3	0	0.1	0.16
5. Progranulocytes	0.3	0.9	1.4	1.1	0.2	0.78
6. N. Myelocytes	29.2	16.4	7.2	23.9	24.4	20.22
7. Immature Mc.	1.1	2.2	1.8	0	0.2	1.06
8. Half-mature Mc.	7.3	4.5	4.4	5.8	1.9	4.78
9. Mature Mc.	20.8	9.7	1.0	18.1	22.3	14.38
10. N. Metamyelocytes	8.5	3.5	2.4	11.3	3.4	5.82
11. N. Band Cells	7.7	4.1	6.6	3.4	4.8	5.32
12. N. Segmented Cells	10.9	28.9	33.3	8.3	17.0	19.68
13. Eosinophils	1.2	1.7	1.6	1.6	1.6	1.54
14. Basophils	0	0.2	0	0	0	0.04
15. Monocytes	0.1	0.6	0.4	0.3	0.4	0.36
16. Lymphocytes	0.2	0.4	0.8	2.1	1.0	0.90
17. Megakaryocytes	0.1	0.1	0.2	0.1	0.2	0.14
18. Plasmocytes	0	0.3	0.3	0	0	0.12
19. Reticulo-endothelial Cells	0.3	0.3	0	0	0	0.12
20. Unclassified Cells*	0	0	0.1	0.1	0	0.04
21. Disintegrated Cells	0	0	0	0	0	0
22. Fatt Cells	0	0	0	0	0	0

* Small lymphocyte-like cells.

Table 7. Bone marrow differential cell count in guinea-pigs that received total body X-irradiated with a dose of 550 r. 10 or 7 days after irradiation.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4*	No. 5*	No. 6*	Mean
1. Proerythroblasts	0	0.5	0.1	0	0	0	0.10
2. Macroblasts	0	1.0	0.2	1.4	0	0.1	0.45
3. Normoblasts	0.6	30.5	7.4	9.6	1.1	8.0	9.53
4. Myeloblasts	0	0.2	0	0.1	0	0	0.05
5. Progranulocytes	0	0.5	0.2	0.2	0	0	0.15
6. N. Myelocytes	1.4	8.7	3.8	7.7	5.4	8.2	5.87
7. Immature Mc.	0	0.9	0	0.2	0	0.2	0.22
8. Half-mature Mc.	0.5	1.8	0.4	0.5	0.4	0.6	0.70
9. Mature Mc.	0.9	6.0	3.4	6.9	5.0	7.4	4.93
10. N. Metamyelocytes	0	0.4	0.2	3.3	0.3	2.8	1.17
11. N. Band Cells	0	0.1	0.4	4.0	0.1	0.6	0.87
12. N. Segmented Cells	0.6	4.1	2.1	25.1	3.0	5.8	6.32
13. Eosinophils	0.2	0	0.4	0.6	0.2	0.1	0.25
14. Basophils	0	0.2	0.2	0	0	0	0.67
15. Monocytes	0	0.2	0	0.2	0.1	0.2	0.12
16. Lymphocytes	0	0.8	0.6	1.3	0.3	3.0	1.0
17. Megakaryocytes	0	0.1	0	0	0	0	0.02
18. Plasmocytes	0	0	0	0.1	0.2	0.6	0.15
19. Reticulo-endothelial Cells	0	0.5	0	0	0.4	0	0.15
20. Unclassified Cells**	0.2	1.6	0.8	0.4	0.4	1.0	0.73
21. Disintegrated Cells	95.6	48.4	77.2	42.5	84.7	67.7	69.35
22. Fatt Cells	1.4	2.3	6.4	3.6	2.8	0.4	2.82

* 7 days after irradiation. ** Small lymphocyte-like cells.

Table 8. Bone marrow differential cell count in guinea-pigs that received a transfusion of bone marrow cells immediately after total body X-irradiation with a dose 550 r. 10 days after transfusion.

Cell count in per cent \ Animal No.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Mean
1. Proerythroblasts	0.9	1.0	0.5	0.2	0.1	0.5	0.53
2. Macroblastss	4.8	3.1	1.4	1.1	1.4	2.2	2.33
3. Normoblasts	51.8	67.8	48.5	46.9	12.1	33.5	43.43
4. Myeloblasts	0.3	0.2	0.2	0.1	0	0.3	0.18
5. Progranulocytes	0.4	0.2	0.3	0.1	0.2	0.5	0.28
6. N. Myelocytes	16.6	12.9	15.7	9.1	15.7	16.2	14.37
7. Immature Mc.	0.5	0.4	0.4	0.1	0.2	0.5	0.35
8. Half-mature Mc.	2.7	1.8	2.3	1.5	1.5	2.9	2.12
9. Mature Mc.	13.4	10.7	13.0	7.5	14.0	12.8	11.90
10. N. Metamyelocytes	2.4	2.0	3.5	0.9	1.1	4.4	2.38
11. N. Band Cells	4.3	2.3	2.2	2.1	2.2	12.8	4.32
12. N. Segmented Cells	9.5	5.2	23.7	27.3	25.3	23.4	19.07
13. Eosinophils	0.9	0.7	0.7	0.3	1.1	0.7	0.73
14. Basophils	0.2	0.7	0.6	0.1	0.1	0.5	0.37
15. Monocytes	0	0	0	0.1	0.1	0.2	0.07
1.6 Lymphocytes	0	0.2	0.7	0.3	0.7	0.2	0.35
17. Megakaryocytes	0.1	0	0.6	0.1	0.4	0	0.20
18. Plasmocytes	0	0	0	0	0	0	0
19. Reticulo-endothelial Cells	0.1	0	0.2	1.2	0.6	1.2	0.55
20. Unclassified Cells*	0	0	0.3	0	1.6	0	0.32
21. Disintegrated Cells	7.7	3.7	0.9	9.8	37.2	3.3	10.43
22. Fatt Cells	0	0	0	0.3	0.2	0	0.08

* Small lymphocyte-like cells.

Table 9. Myelopoiesis in the spleen of X-irradiated guinea-pigs, 10 days after transfusion of intact, damaged, and heterologous marrow cells.

Transfused cells	Slight degree of myelopoiesis		Moderate degree of myelopoiesis		Extensive myelopoiesis	
	No./total	per cent	No./total	per cent	No./total	per cent
Guinea-pig marrow cells	1/6	17	3/6	50	2/6	33
Guinea-pig marrow cells damaged by heating	0/6	0	1/6	17	0/6	0
Rabbit marrow cells	0/6	0	2/6	33	0/6	0
Control : without marrow cell transfusion	0/6	0	0/6	0	0/6	0

Thus, there seems to be almost complete recovery of erythropoiesis in the marrow already at 10 days after marrow cell transfusion. Until that time restoration of granulopoiesis appears to be incomplete, however. It was found that while the normal average percentage of myelocytes in the marrow imprints was 20.22 per cent, the corresponding values for the irradiated animals without and with marrow cell transfusion at 10 days were 5.87 per cent and 14.37 per cent respectively.

In brief, it can be stated that in the process of hematopoietic recovery after transfusion of marrow cells into irradiated animals, the restoration of erythropoiesis preceeds that of granulopoiesis in both the spleen and bone marrow.

Finally, the author wishes to call attention to the finding that, as shown in Table 2, the degree of hematopoietic recovery after marrow cell transfusion appeared to be greater in the females than in the males. This finding is very important, but it requires further investigation to make sure that there is a sex difference in the process of hematopoietic recovery in X-irradiated animals after marrow cell transfusion.

B. Transfusion of damaged and heterologous bone marrow cells

Ectopic myelopoiesis in the spleen and regeneration of bone marrow was also elicited by transfusion of guinea-pig marrow cells damaged by heating at 50°C. for 30 minutes as well as by transfusion of rabbit marrow cells. In such instances, however, hematopoietic recovery occurred to a much lesser degree than with transfusion of intact guinea-pig marrow cells (cf. Tables 9 and 10).

V. DISCUSSION

Since in the rabbit the special leukocytes have distinct pseudoeosinophilic granules, the appearance of the transfused immature forms of this series can easily be recognized in foreign situations, in which these cells otherwise do not occur. The previous experiments on normal rabbits by *Osogoe* and *Omura* (1950) have shown that after transfusion of a large amount of bone marrow cells, chief accumulation of the

Table 10. Regeneration of bone marrow of X-irradiated guinea-pigs, 10 days after transfusion of intact, damaged, and heterologous marrow cells.

Transfused cells	Slight degree of regeneration		Moderate degree of regeneration		Extensive regeneration	
	No./total	Per cent	No./total	Per cent	No./total	Per cent
Guinea-pig marrow cells	0/6	0	2/6	33	4/6	67
Guinea-pig marrow cells damaged by heating	1/6	17	0/6	0	1/6	17
Rabbit marrow cells	0/6	0	2/6	33	0/6	0
Control : without marrow cell transfusion	0/6	0	0/6	0	0/6	0

injected immature cells of both the granulocytic and the erythrocytic series takes place in the liver and spleen. Although the special granulocytes of the guinea-pig also have distinct pseudoeosinophilic granules like those of the rabbit, it was very difficult to find the transfused marrow cells in the liver, spleen and other organs of the recipient guinea-pigs. Nevertheless, after transfusion of bone marrow cells labelled with radioactive phosphorus (P^{32}) into X-irradiated guinea-pigs, the radioactivity per gram of fresh tissue was found to be far greater in the spleen and bone marrow than in other organs. This was not the case in the control animals injected with comparable amounts of inorganic P^{32}. The results indicate that chief accumulation of the injected marrow cells occurred in the spleen and bone marrow. The occurrence of the transfused marrow cells labelled with S^{35}-methionine in the bone marrow of rats lethally irradiated with X-rays has been directly demonstrated by *Odell* and *Smith* (1958) by the use of radioautography.

It is important to point out here that the injected marrow cells accumulate in the bone marrow to a great extent, when this tissue has been severely damaged by a large dose of total-body X-irradiation before injection. This does not appear to occur after transfusion of bone marrow cells into normal animals. In such instances, chief accumulation of the injected marrow cells takes place in the liver and spleen, as previously reported by *Osogoe* and *Omura* (1950).

The point of primary interest concerns the proliferation of the marrow cells of the donors accumulated in the spleen and bone marrow of the recipients. Extensive myelopoiesis in the spleen and rapid regeneration of bone marrow after transfusion of marrow cells into irradiated guinea-pigs give substantiating evidence for the active proliferation of the accumulated marrow cells in these organs. Of particular interest in this connection is the fact that in the process of hematopoietic recovery after transfusion of marrow cells into irradiated guinea-pigs, the restoration of erythropoiesis preceeds that of granulopoiesis in both the spleen and bone marrow. Since,

as already mentioned, the marrow elements which accumulated in the liver, spleen and other organs after bone marrow transfusion were unable to continue to proliferate for a long time when normal animals were used as recipients (*Osogoe* and *Omura*, 1950), it is indisputable that severe radiation injury of the host animal's bone marrow facilitates the further proliferation of the transfused marrow cells accumulated in the spleen and bone marrow.

From the above considerations it may be concluded that colonization of the transfused marrow cells and their proliferation in the spleen and bone marrow may contribute to repopulation of myeloid tissue in X-irradiated animals.

Finally, a brief mention should be made of the bone marrow therapy in radiation injury, which has been initially developed by *Lorenz* et al. (1951). On the basis of innumerable experiments on mice, rats, guinea-pigs, dogs and monkeys, it is now generally believed that transfusion of the cellular elements of bone marrow is followed by colonization of the host animal's bone marrow by donor cells (for reviews cf. *Lorenz* and *Congdon*, 1954; *Bond* and *Cronkite*, 1957; *Congdon*, 1957 and 1959). *Jacobson* and his associates maintain, on the other hand, that there are some nonspecific factors in the spleen and bone marrow which enhance hematopoietic recovery from radiation injury. In view of the present finding that hematopoietic recovery from radiation injury was also elicited by transfusion of homologous cells damaged by heating at 50°C. for 30 minutes as well as by transfusion of heterologous marrow cells, the author also agrees with the view of *Jacobson* and others. It seems quite reasonable to consider these factors to be secondary phenomena that cause the colonized marrow cells to grow in the bone marrow of the irradiated recipients.

VI. SUMMARY

1. A saline suspension of bone marrow from femur, tibia and humerus of 2 to 7 adult guinea-pigs was injected intravenously into another adult guinea-pig that had been irradiated with a lethal dose of X-rays on the whole body (550 r). In order to clarify the pattern of the initial distribution of the injected cells, the bone marrow elements to be transfused were labelled with radioactive phosphorus (P^{32}) by injecting it into the donor guinea-pigs 48 hours previously.

2. The amounts of radioactivity recovered per gram of fresh tissue and per organ indicated that chief accumulation of the transfused marrow cells occurred in the spleen and bone marrow.

3. On the 5th day after the marrow cell transfusion, active proliferation of myeloid cells, erythroblasts in particular, began to take place in the spleen. This was accompanied by a slight degree of regeneration of bone marrow. By the 10th day, the activity of myelopoiesis in the spleen was greatly intensified and extensive regeneration of bone marrow was observed. In the control animals irradiated with X-rays without marrow cell transfusion, on the other hand, there was no indication

of hematopoietic recovery either in the spleen or in the bone marrow until the 10th day; both organs were almost completely depleted of proliferating cells.

4. In the process of hematopoietic recovery after transfusion of bone marrow cells into irradiated animals, the restoration of erythropoiesis preceeded that of granulopoiesis in both the spleen and the bone marrow.

5. Ectopic myelopoiesis in the spleen and regeneration of bone marrow were also elicited by transfusion of guinea-pig marrow cells damage by heating at 50°C. for 30 minutes as well as by transfusion of rabbit marrow cells. In such instances, however, hematopoietic recovery occurred to a much lesser degree than with transfusion of intact guinea-pig marrow cells.

6. The results obtained give evidence to substantiate that severe radiation injury of the host animal's bone marrow facilitates the proliferation of the transfused marrow cells accumulated in the spleen and bone marrow. At the same time it can be stated that colonization of the transfused marrow cells and their proliferation in the spleen and bone marrow may contribute to repopulation of myeloid tissue in X-irradiated animals.

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