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Effect of Bleeding on Irradiation-Induced Erythropoiesis in the Spleen of AKR Mice

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The effect of erythropoietin, increased by bleeding, on the erythropoiesis induced by irradiation in the spleen of AKR mice, has been studied. The following parameters were measured to quantify the erythropoietic activity: the number and size of hematopoietic nodules (colonies) and proerythroblasts in the spleen, the spleen, blood and red-cell ^{59}Fe uptake and the hematocrit and reticulocytes in the blood.

Under erythropoietic stimulus an increase in the number and size of colonies was observed and these colonies were observed sooner because of their more rapid growth. The proerythroblasts in the spleen appeared earlier, and there were increases in the spleen, blood and red-cell ^{59}Fe uptake and in the hematocrit and reticulocytes in the blood.

The erythropoietic process consists in a cellular proliferation, forming red-cells from stem-cells (4, 6). This process is mainly regulated by the erythropoietin (3, 14) but the precise mechanism by which this hormone regulates erythropoiesis is not known.

The aim of this work is to study the effect of the erythropoietin, induced by bleeding, on the different steps of irradiation-induced erythropoiesis in the spleen of mice.

Under the irradiation, each stem-cell is activated leading to a cellular clone which forms a colony appearing as nodules in the surface of the spleen (11). In this paper the erythropoietic activity of the spleen has been measured morphologically and functionally in anemic mice by the count of proerythroblasts in the spleen, by the number and size of the hematopoietic colonies in the spleen, by histological cuts, and by the ^{59}Fe uptake by the spleen. The ^{59}Fe uptake and reticulocytes in the blood, and the hematocrit, have also been measured.

Materials and Methods

The mice were syngeneic AKR of 23 ± 1 g and 8 ± 1 weeks old. Bleeding was performed on 6 consecutive days. Every

day each mouse was bled by puncturing an orbital sinus under light ether anesthesia with a hematocrit tube, and a volume of five such tubes (approximately 0.4 ml of blood) was removed with each flebotomy, in accordance with BOGGS *et al.* (2).

On the sixth day, mice that had been bled and anesthesized, and controls that had not been bled or anesthetized, were simultaneously irradiated (whole body) at 750 R with conventional radiotherapy, as previously described (1).

On day 7 a group of mice, bled and not-bled, were killed. Each mouse had been injected intraperitoneally 16 hours before killing with 0.2 μCi ^{59}Fe, as previously described (9). Other groups were injected and killed in the same manner on day 8, 10, 12 and 14.

The reticulocytes were counted staining peripheric blood with new methylene blue. The erythropoietin was measured by inhibition hemagglutination assay (7, 8). The spleen ^{59}Fe uptake was determined in an LKB Ultrogamma 1280 counter. The separation of the spleen cells was carried out by a gentle maceration, centrifugation, and washing the sediment with isotonic saline solution. After determining the ^{59}Fe uptake, the proerythroblasts were counted after extension and staining with May-Gründwald-Giemsa.

The hematopoietic colonies were counted on the spleen surface using a PZO tridimensional microscope, and their sizes were determined by superposition of a graduated scale.

Results

The anemic mice showed an increase of the seric erythropoietin that began on day 1 and peaked on day 7 (fig. 1).

The spleen colonies (macroscopic) appeared on day 10 in the anemic mice and on day 12 in the control mice. The number of spleen colonies was larger in the anemic mice at all times (fig. 1).

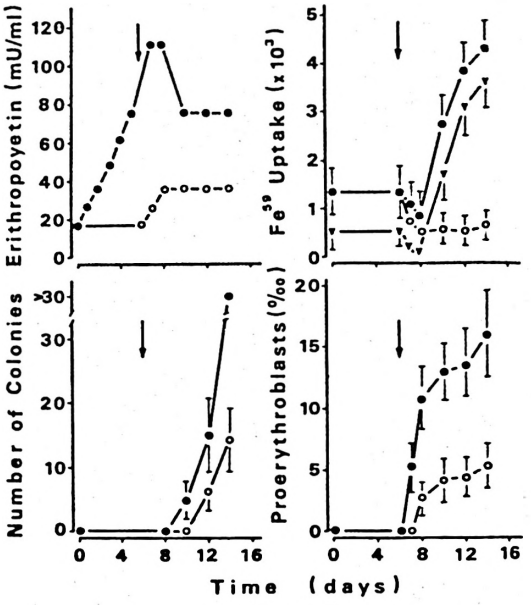

Fig. 1. *Seric erythropoietin, spleen ^{59}Fe uptake, number of spleen colonies and proerythroblasts per thousand nucleated cells in the spleen of AKR mice irradiated on day 6 (arrow), bled on days 0, 1, 2, 3, y and 5 (●) and not-bled (○).*
Also the spleen intracellular ^{59}Fe uptake in bled and irradiated mice has been measured (▲). Ten mice were killed for each data point. The vertical lines represent the standard deviation.

The mean colony diameter measured on day 14 was larger in the anemic mice (table I).

Both the total spleen ^{59}Fe uptake and ^{59}Fe uptake in the separate spleen cells were larger in the anemic animals, and this difference increased with the time after irradiation (fig. 1).

The proerythroblasts appeared in the spleen of the anemic mice on day 7 (24 hours after irradiation). In the control animals they appeared on day 8 (48 hours after irradiation). The number of proerythroblast were, at all times, larger in the anemic mice (fig. 1).

After irradiation a much quicker recuperation of hematocrit, and an increase

Table I. *Size (mm) of colonies on day 14 on the spleen of AKR irradiated+ mice.* Each value represents the mean ± S.D. of 40 colony diameters.

Bled++	2.52 ± 0.68
Not bled	1.72 ± 0.69

* On day 6. ** On days 0, 1, 2, 3, 4 and 5.

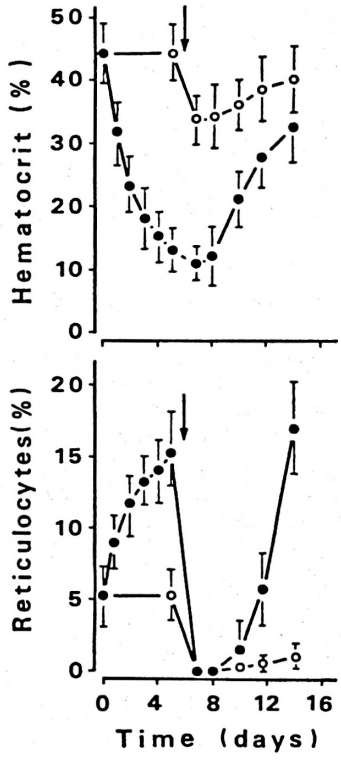

Fig. 2. *Hematocrit and reticulocytes in mice irradiated on day 7 (arrow), bled on days 0, 1, 2, 3, 4 and 5 (●) and not bled (○).* Ten mice were killed for each data point. The vertical lines represent the standard deviation.

of reticulocytes in the blood, was observed in the anemic mice relative to the controls (figure 2).

Total blood ^{59}Fe uptake and red cell ^{59}Fe uptake were larger in the anemic mice (fig. 3). The anemic mice that were splenectomized before ^{59}Fe injection showed a total blood ^{59}Fe uptake lower than non-operated anemic mice (fig. 3).

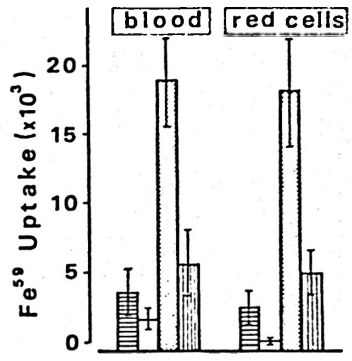

Fig. 3. *^{59}Fe uptake by blood an by the isolated red-cells on day 14.*
The mice were bled on days 0, 1, 2, 3, 4 and 5, irradiated on day 6 and splenectomized on day 14. Control animals: normal (horizontal-lined bar), irradiated (white bar), bled and irradiated (dotted-bar). Problem animals: bled, irradiated and splenectomized (vertical-lined bar). Each bar is the mean value for ten mice, and the vertical lines represent the standard deviation.

Discussion

In agreement with TILL and MCCULLOCH's (12) and TRENTIN's (13) demonstration that each colony proceeds from the proliferation and maturation of one stem-cell, the increase in the number of colonies observed in the spleen of the anemic mice (fig. 1) suggests that under the erythropoietic stimulus produced by bleeding, a greater number of stem-cells complete their erythroid differentiation up to the point of originating macroscopic colonies in the spleen.

The increase in size of colonies in the anemic mice (table I) may be explained as a result of an acceleration in cell proliferation due to erythropoietin. This aspect has been verified studying histological sections of the spleen.

In the anemic mice, the stem-cell covers the different maturing stages which separate it from the proerythroblast in 24 hours, while in the non-bled mice this process takes 48 hours (fig. 1). This fact demonstrates the stimulating effect of erythropoietin on the cell maturation in stages previous to the proerythroblast.

The arrival of proerythroblasts at the erythron, being earlier and more intense in the anemic mice, must produce in them an increase in those parameters that are the expression of the functional capacity of this cellular compartment. The result obtained in measuring hematocrit, reticulocytes, total blood ^{59}Fe uptake, and red-cell ^{59}Fe uptake, confirms this hypothesis (figs. 2 and 3).

The increase in the functional capacity of the erythron has its expression in the spleen. The increase of the ^{59}Fe uptake by the spleen of anemic mice (fig. 1) indicates an increase in their erythropoietic activity. Although this could be a result of a non-specific ^{59}Fe uptake, this possibility has been discarded, since the ^{59}Fe uptake of the spleen cellular fraction is very similar to the total spleen ^{59}Fe uptake.

Both the total blood ^{59}Fe uptake and red-cell ^{59}Fe uptake are lower in anemic mice that have been splenectomized before injecting ^{59}Fe than in anemic mice that have not been operated. This indicates that the participation of the bone marrow in these parameters is about 25 % of that of the spleen.

In this work the problem animals were anesthetized with ether. In accordance with the works performed by FRIED (5) and PESCHLE *et al.* (10) this anesthetic did not appear to affect the results obtained.

Resumen

Se estudia el efecto de la eritropoyetina, aumentada por sangría, sobre la eritropoyesis inducida en el bazo de ratones AKR por irradiación. La actividad eritropoyética se cuantifica a través de la medida de los siguientes parámetros: número y tamaño de nódulos hematopoyéticos (colonias) y proeritroblastos en bazo, captación de Fe^{59} por bazo, sangre y hematíes y valor hematocrito y reticulocitos en sangre.

Se observa un incremento en el número y tamaño de las colonias y un acortamiento en el tiempo de aparición de éstas. Igualmente se aprecia aparición precoz de proeritroblastos en bazo, incremento de la captación de Fe^{59} por el bazo, sangre y hematíes y aumento del valor hematocrito y reticulocitos en sangre.

References

1. BAJO, R., ALONSO, A. and PEÑA-MARTÍNEZ, J.: *Rev. esp. Fisiol.*, **35**, 161-164, 1979.
2. BOGGS, D. R., MARSH, J. C., CHERVENICK, P. A., CARTWRIGHT, G. E. and WINTROBE, M. M.: *Rad. Res.*, **35**, 68-77, 1968.
3. CARNOT, P. and DEFLANDRE, G.: *Comp. Renal Acd. Sci.*, **143**, 284-386, 1906.
4. FISHER, J. W.: Kidney Hormones. Vol. 2. Academic Press. London, 1977.
5. FRIED, W.: *Blood*, **40**, 671-677, 1972.
6. HARRISON, P. R.: *Nature*, **262**, 353-356,
7. LANGE, R. D., MCDONALD, T. P. and JORDAN, T. A.: *Lab. Clin. Med.*, **73**, 78-90, 1969.
8. LANGE, R. D., MCDONALD, T. P., JORDAN, T. A., TROBAUGH, R. E. Jr., KRETCHMAR, A. L. and CHERNOFF, A. E.: In «Hematopoietic cellular proliferation». (Stohlman, F. Jr., ed.). Grune and Stratton, New York, 1970, pp. 122-132.
9. PEÑA-MARTÍNEZ, J., HUBBER, B. and FESTENSTEIN, H.: *Transp. Proc.*, **5**, 1393-1397, 1973.
10. PESCHLE, C., SASSO, G. F., RAPPAPORT, I. A. and CONDORELLI, M.: *J. Lab. Clin. Med.*, **79**, 950-959, 1972.
11. TILL, J. E. and MCCULLOCH, E. A.: *Rad. Res.*, **14**, 213-222, 1961.
12. TILL, J. E. and MCCULLOCH, E. A.: *Rad. Res.*, **18**, 96-105, 1963.
13. TRENTIN, J. J.: In «Regulation of Hematopoiesis», I (A. S. Gordon, ed.). Appleton-Century-Crofts, New York, 1970, p. 159.
14. WHITE, W. F. and TOSH, G.: *Proc. Soc. Exp. Biol.*, **102**, 686-690, 1959.