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EFFECTS OF RADIATION AND HYPOXIA ON THE METABOLIC FATE OF ERYTHROPOIETIN

by

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The metabolic fate of erythropoietin is poorly understood. STOHLMAN & BRECHER (1959) suggested that plasma levels of erythropoietin depended upon the state of bone marrow function. NAETS & WITTEK (1968, 1969), rejected the idea that differentiated erythroid cells influence metabolism; similar results have been reported by BOZZINI (1966). Since both arguments are inconclusive, an investigation into the relationship between the bone marrow cellularity and the circulating erythropoietin in rats submitted to hypoxia and ionizing radiations was performed.

Material and Methods. Six groups of male Sprague-Dawley rats, 10 to 12 weeks old, were investigated: (1) twenty normal rats at sea level, (2) twenty rats submitted for 24 hours to 19 000 feet, (3) twenty-four rats irradiated with 200 R whole body irradiation and kept at 19 000 feet for 24 hours, (4) twenty-four rats irradiated with 200 R and sacrificed 24 hours later (at sea level), (5) twenty rats kept at 19 000 feet for seven days, and (6) twenty rats kept at 19 000 feet for seven days and irradiated with 200 R on the sixth day of hypoxia (see Fig. 1).

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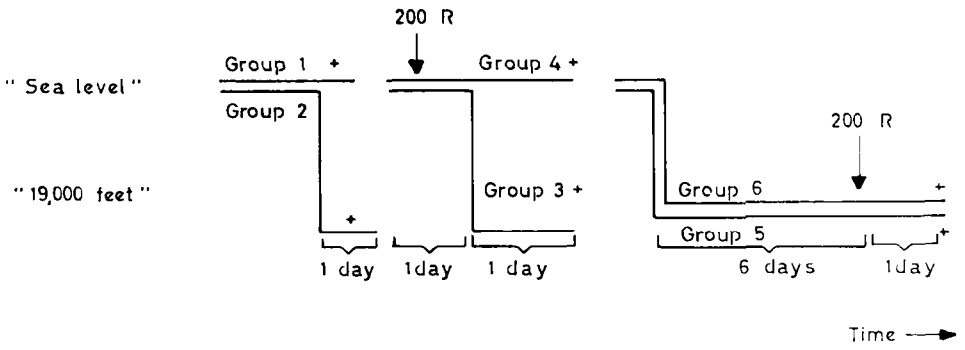

Fig. 1. Working scheme. + : day of sacrifice.

Rats exposed to hypoxia for seven days were kept in the barometric chamber for 23 hours daily; one hour was reserved to clean the cages and change the food and water. The animals were sacrificed by cardiac puncture under ether. The following controls were performed, hematocrit, reticulocyte, hemoglobin, red blood cell counts with a Coulter Counter Model B and bone marrow: femur smears were made by the brush technique and no less than one thousand nucleated cells were counted. The spleens were weighted, imprints were made and no less then one thousand cells were counted. The plasma of these animals was separated by centrifugation at 3 000 rpm for 30 minutes in a cold centrifuge. The fasting rat test was used: Sprague-Dawley female rats, 8 to 12 weeks old were kept without food and at the second and third day of fasting two ml/day/rat injected subcutaneously and on the fourth day 0.5 μCi ^{59}Fe introduced intravenously. The animals were sacrificed 24 hours later by cardiac puncture under ether. Hematocrit, reticulocyte and ^{59}Fe incorporation in the red blood cells tests were performed, assuming a blood volume of 5 ml per 100 g body weight. The erythropoietic activity of the injected plasmas was expressed in units of erythropoietin per ml, withdrawn from a dose-response curve of erythropoietin Standard B.

Results

The peripheral values of the different groups indicated an increase in hematocrit, reticulocyte and hemoglobin values after seven days of hypoxia (Table 1). Reticulocytes were significatively reduced in the irradiated groups. The percentage of erythroblasts in bone marrow and spleen also followed this pattern

Table 1

Hematocrit, hemoglobin and reticulocyte values in rats submitted to hypoxia alone, irradiation alone and hypoxia and irradiation. Hx: hypoxia, (±): standard error.

Groups	Hematocrit %	Hemoglobin g %	Reticulocytes (mm^3) $\times 10^5$
1 (Normals)	40.3±0.5	12.3±0.16	2.08±0.13
2 (24h Hx)	44.3±0.9	14.4±0.3	3.06±0.31
3 (24h Hx + 200R)	42.5±0.6	13.1±0.2	1.54±0.25
4 (Sea level 24h + 200R)	39.5±0.6	11.4±0.4	0.58±0.15
5 (7d Hx)	53.9±1.0	18.2±0.4	4.51±0.36
6 (7d Hx + 200R)	54.5±1.0	18.1±0.2	2.12±0.39

Table 2

Erythroblasts in bone marrow and spleen with spleen weights. Hx: hypoxia, (±): standard error.

Groups	Erythroblasts (%)		Spleen weight (g)
	Bone marrow	Spleen	
1 (Normals)	24.7±1.5	3.5 ±0.5	0.637±0.05
2 (24h Hx)	26.5±1.8	2.4 ±0.5	0.498±0.06
3 (24h Hx+200 R)	10.0±1.9	1.0 ±0.4	0.298±0.03
4 (Sea level 24h+200R)	6.7±1.1	0.17±0.13	0.342±0.01
5 (7d Hx)	33.3±1.3	5.7 ±0.8	0.672±0.09
6 (7d Hx+200R)	14.6±2.0	1.3 ±0.4	0.257±0.02

Table 3

Units of erythropoietin per ml of plasma injected subcutaneously in fasting rats. Hx: hypoxia, (±): standard error.

Groups	Units of erythropoietin per ml of plasma
1 (Normals)	0
2 (24h Hx)	2.1±0.5
3 (24h Hx+200R)	4.0±0.8
4 (Sea level 24h+200R)	0
5 (7d Hx)	0
6 (7d Hx+200R)	2.2±0.5

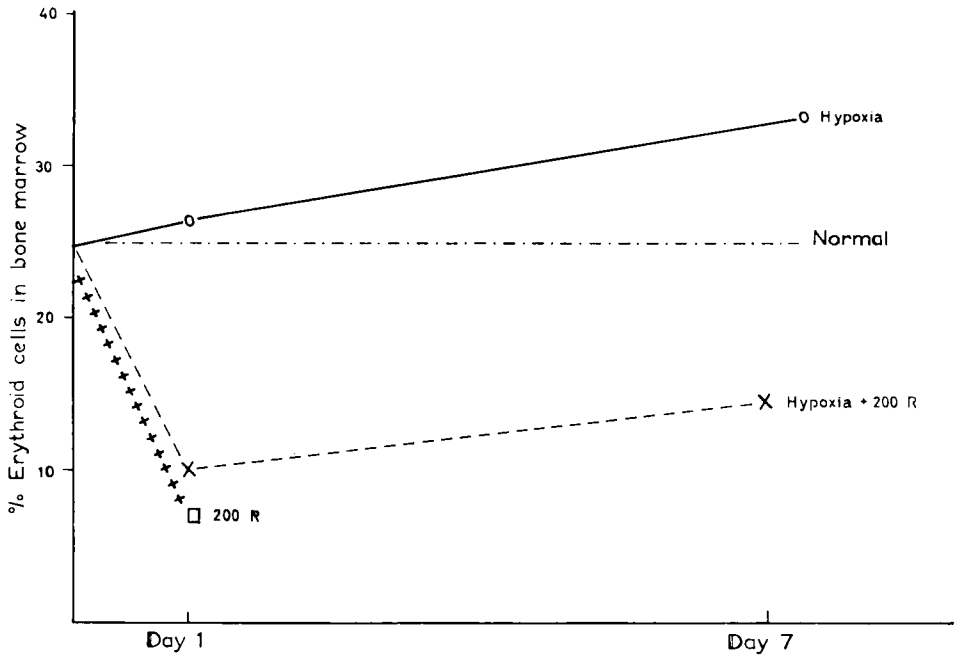

Fig. 2. Percentage of erythroid cells in bone marrow in rats subjected to hypoxia alone, irradiation alone and hypoxia and irradiation.

(Table 2). The size of the spleens was reduced in the irradiated groups. After 24 hours of hypoxia, 2.1 units of erythropoietin were present, but at the seventh day the plasma revealed no erythropoietic activity (Table 3). The irradiated animals in hypoxia increased in erythropoietic plasma content.

Discussion

Hypoxia induces an increase in bone marrow erythroid precursors that is followed by reticulocytosis (Figs 2, 3). Two hundred R reduce the percentage of erythroblasts in bone marrow even in the presence of the hypoxic stimulus (Figs 2, 3 and Table 2). Circulating erythropoietin is increased twofold at one and 7 days of hypoxia in irradiated rats (Fig. 4). The author was unable to demonstrate erythropoietin at the seventh day of hypoxia in non-irradiated animals. This was also observed in the urines of human subjects exposed to 13 127 feet (CARMENA 1964) and 14 900 feet (CARMENA et coll. 1967).

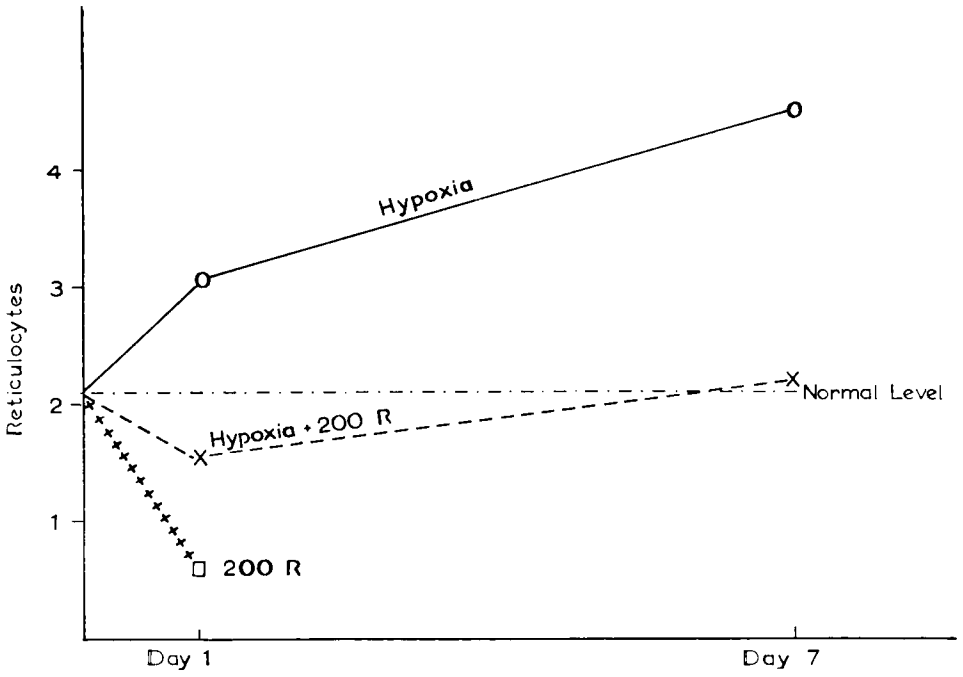

Fig. 3. Number of reticulocytes per mm^3.

The results reinforce the hypothesis of STOHLMAN & BRECHER (1959) in the sense that erythropoietin is used or degraded by the erythroid tissue. This hypothesis is also supported by the works of HAMMOND & ISHIKAWA (1962) who demonstrated that the rate of disappearance of erythropoietin from the plasma was faster in patients with hemolytic anemia than in those with hypoplastic anemia after blood transfusion. JACOBSON et coll. (1959) were of the same opinion. The author has detected erythropoietin in the plasma of dwellers at 14 900 feet who descended to sea level at up to 42 hours, due probably to a failure in the utilization of the hormone by hypoplastic bone marrow. The contradictory results of NAETS & WITTEK (1968, 1969) and BOZZINI (1966) could be due to the animal employed. The bone marrow of the dog is not affected by hypertransfusion as in other mammals (STOHLMAN) and at 14 000 feet does not respond to hypoxia unless the animal is subjected to exercise (PACE).

It would appear that the presence of erythropoietin in plasma is in inverse relationship to the cellularity of the erythroid bone marrow.

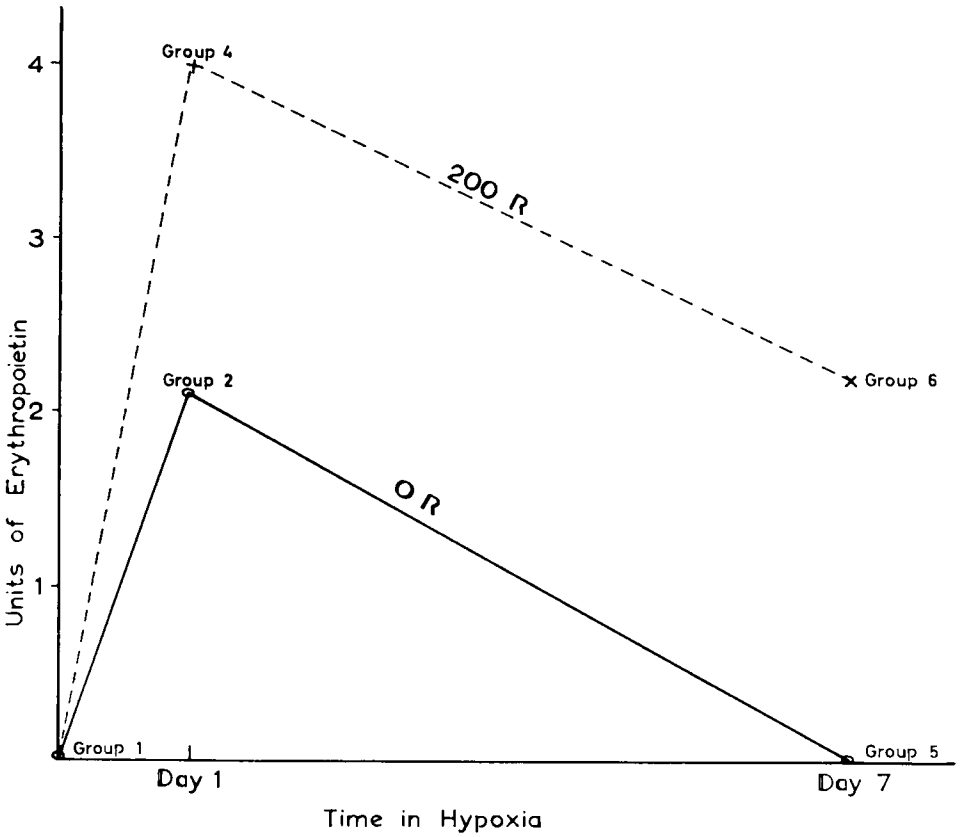

Fig. 4. Units of erythropoietin per ml of plasma injected in fasting rats.

Acknowledgement

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SUMMARY

Sprague-Dawley rats were kept at 19 000 feet for various periods. The animals that received 200 R whole body irradiation had a significantly increased plasma erythropoietin. It is concluded that the levels of erythropoietin are in inverse relationship to the cellularity of the erythroid precursors.

ZUSAMMENFASSUNG

Sprague-Dawley Ratten wurden verschiedene Zeit lang in 19 000 Fuss gehalten. Die Tiere, die eine Ganzkörperbestrahlung von 200 R erhalten hatten, hatten einen signifikanten Anstieg des Plasmaerythropoietins. Es wird gefolgert, dass der Erythropoietinspiegel in einem umgekehrten Verhältnis zur Zellularität der erythroiden Präkusoren steht.

RÉSUMÉ

Des rats Sprague-Dawley ont été maintenus à 19 000 pieds pendant différentes périodes. Les animaux qui ont reçu une irradiation totale du corps de 200 R ont présenté une élévation significative de l'érythropoïétine plasmatique. L'auteur conclut que le taux d'érythropoïétine est en relation inverse du nombre des cellules des précurseurs érythroïdes.

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