Presence of Plasma Erythropoietin in Hypoxic Rats with or without Kidney(s) and/or Spleen. (23390)

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The presence of an erythropoietic factor in the plasma of animals subjected to hypoxic hypoxia has been reported recently by Stohlman(1) and Prentice and Mirand(1a). Additional data from our laboratory bear out a time-concentration relationship of plasma erythropoietin (EPF) in hypoxic hypoxic rats. Knowing of this time-concentration of EPF, studies were carried out to determine whether this time-concentration relationship of plasma EPF can be altered in absence of the kidney(s) and/or the spleen. Jacobson *et al.*(2) reported recently that bleeding and cobalt chloride administration did not produce circulating erythropoietin in the absence of the kidneys and postulated that the kidney is the site of production of EPF. The purpose of this report is to demonstrate that hypoxic hypoxic rats with or without their kidney(s) and/or spleen can produce erythropoietin.

Material and methods. Sprague-Dawley rats were placed in chambers constructed of plexiglass. Into each chamber was directed a mixture of nitrogen and oxygen, the proportions of which, along with ambient pressure, determined the simulated altitude. Flow of nitrogen and oxygen was regulated by a rotameter type of flow-meter and remained quite constant over the experimental period. The chamber O_2 concentration was checked by reading of the oxygen and nitrogen flowmeters and also by a portable Beckman Oximeter. Random air samples were also taken from the chamber in a Douglas bag and checked with a mass spectrograph for O_2, N_2 and CO_2. Good agreement in results was obtained. A flow of approximately 5 liters/minute was maintained through the chamber and soda lime and sulfuric acid were placed in the chamber to minimize CO_2 and humidity levels.

Sprague-Dawley rats, 2 to 3 months old, used as donors, were exposed in a chamber to an atmosphere of approximately 10% O_2 for the time intervals shown in Table I. Rats of another group, used as donors, were nephrectomized and/or splenectomized and caution was taken during the operation that no bleeding occurred. They were then immediately placed in an atmosphere of 10% O_2 at various time intervals (Table II). Food (Purina Laboratory chow) and H_2O were available to rats in the chamber at all times. At the end of the respective intervals the donor rats were withdrawn from the chamber and promptly exsanguinated from the dorsal aorta. A final hematocrit was done on each rat and no animal with hematocrit below 40 was used as a source of plasma. Plasma obtained was frozen and stored until used.

Recipient animals were 3-month-old Sprague-Dawley rats which were used as assay animals approximately 15-20 days after hypophysectomy(3,4). They received 2 cc of donor rat plasma to be tested I.V. via the jugular vein on 2 successive days. On day 3 approximately 1 μc Fe^{59} was given I.V. via femoral vein and on day 4, a 24-hour Fe^{59} uptake was done. The results for each group of animals were expressed as the average 24-hour Fe^{59} uptake for the particular group.

Results. The time-concentration relationship of plasma EPF in hypoxic hypoxic rats with kidneys and spleen intact is clear cut (Table I). The concentration is approxi-

TABLE I. Hypophysectomized Rats (Recipient) Receiving Plasma from Normal Rats (Donor) Kept in 10% Oxygen for Varying Periods of Time. Erythropoiesis in recipient as judged by Fe^{59} uptake.

Treatment of donor rats at 10% O_2 atmosphere at various intervals (hr)	No. of recipient rats	24 hr Fe^{59} uptake
4	4	15.6 ± 5.88*
8	4	32.0 ± 4.20
24	5	34.5 ± 7.7
48	5	2.6 ± 1.8
120	6	2.6 ± 1.4
0 (normal plasma)	4	2.7 ± 1.86
0 (untreated)	3	3.3 ± 1.85

* ± S.D.

TABLE II. Hypophysectomized Rats (Recipient) Receiving Plasma from Normal Sprague-Dawley Rats (Donor) Which Were Nephrectomized and Splenectomized and Kept in 10% Oxygen for Varying Periods of Time. Erythropoiesis in recipient as judged by Fe^{59} uptake.

Treatment of donor rats prior to placing in 10% O_2 atmosphere	Interval at 10% O_2	No. of recipient rats	24 hr Fe^{59} uptake
Unilateral nephrectomy	4	5	13.9 ± 4.13*
	8	5	31.2 ± 5.84
	24	6	40.0 ± 5.66
Bilateral nephrectomy	4	6	17.9 ± 3.06
	8	5	13.2 ± 4.23
	24	6	37.9 ± 2.53
Sham nephrectomy	4	6	21.8 ± 4.84
	8	5	29.1 ± 6.41
	24	6	35.4 ± 7.70
Splenectomy	4	3	25.1 ± 1.70
	8	5	33.3 ± 6.37
Splenectomy-unilateral nephrectomy	4	6	21.8 ± 5.37
	8	5	37.8 ±11.45
Splenectomy-bilateral nephrectomy	4	4	13.4 ± 5.36
	8	6	17.6 ± 1.91
Sham splenectomy-nephrectomy	4	6	22.4 ± 5.12
	8	6	35.1 ± 4.78
Normal rat plasma	—	5	7.6 ± 2.69
Untreated	—	6	6.7 ± 4.0

* ± S.D.

Assays in all groups were carried out at the same time and under similar circumstances. In no instance did similar treated rats at normal atmosphere show a significant elevation of erythropoietin titers.

mately 5 times the control level at 4 hours. At 8 hours the titer reaches 10 times control level and remains approximately the same at 24 hours. By 48 and 120 hours the titer has dropped back to control levels. The time-concentration relationship of plasma EPF in unilateral and bilateral nephrectomized animals subjected to hypoxic hypoxia is doubled at 4 hrs; doubled or quadrupled at 8 hrs, and 5-6 times greater at 24 hrs than the titers at control levels (Table II). Splenectomized, and splenectomized-unilaterally nephrectomized and splenectomized-bilaterally nephrectomized rats subjected to hypoxic hypoxia all show similar increases in EPF titers of about the same magnitude as that shown in the nephrectomized rats.

Nephrectomized and/or splenectomized rats exposed to normal atmosphere did not show any elevation of EPF. Their Fe^{59} values were comparable to values reported in Table II for hypophysectomized rats receiving normal plasma and those that were untreated.

Discussion. Increasing attention is being paid to the role of plasma erythropoietin in the control of erythropoiesis(6,7,8,9). The erythropoietin level in hypoxic hypoxia, a potent stimulus for the production of erythrocytes in an organism(5,6), is most relevant to the problem of control of erythropoiesis. Our studies indicate that EPF is elevated in the plasma of hypoxic rats during a limited time. This is in contrast to animals made anemic from bleeding or phenylhydrazine. Anemic rats seem to retain elevated levels of EPF in their plasma as long as they have a significant degree of anemia. The reason for this difference is not clear because present knowledge on relative rates of production and utilization or destruction of EPF in hypoxic hypoxia, or other erythropoietic stimulating states is not adequate.

Our studies also bear out that erythropoietin production can go on in the absence of the kidneys and/or spleen in rats subjected to hypoxic hypoxia. This is in contrast to Jacobson's *et al.*(2) and Erslev's(10) data

which indicate that neither rats nor rabbits kept at a normal atmosphere after bilateral nephrectomy have the capacity to respond to a single dose of cobaltous chloride or a single massive hemorrhage by an elevation of plasma erythropoietin. Goldwasser *et al.*(11) recently reported that cobalt enhances red cell production by increasing formation of erythropoietin. Their preliminary report also emphasizes that the erythropoietin in anemic plasma has similarities to erythropoietin in cobalt plasma. It remains to be determined whether there are different erythropoietins in animals and human and whether there are different production sites and different release mechanisms for each. Assuming that there is just one kind of erythropoietin produced, our results then suggest that the kidney and spleen are not the production sites for erythropoietin. Gordon *et al.*(12) attempted, but unsuccessfully, to identify the site of production of circulating erythropoietin by assaying acidified boiled filtrates prepared of plasma, packed blood cells, and various organs. They postulate that the factor may actually be formed in plasma as a result of enzymatic action upon substrates provided by the blood-forming organs.

Summary. (1) Normal rats placed in a low oxygen atmosphere for time periods from 4 to 120 hours reveal definite elevation of plasma erythropoietin until 24 hours and after 48 hours erythropoietin drops back to normal levels as judged by Fe^{59} uptake assay using hypophysectomized rats. (2) On the basis of this time-concentration relationship of plasma erythropoietin in hypoxic hypoxic rats, no significant alteration of erythropoietin titers is evident in hypoxic rats with or without kidneys and/or spleen. (3) Hence, neither the kidney nor spleen is the production site of erythropoietin under hypoxic hypoxia.

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