Title

Erythropoietin Production and Erythrocytic 2,3-Diphosphoglycerate Changes in Normal, Nephrectomized and Hypophysectomized Rats in Hypoxia1

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ERYTHROPOIETIN PRODUCTION AND ERYTHROCYTIC 2,3-DIPHOSPHOGLYCERATE CHANGES IN NORMAL, NEPHRECTOMIZED AND HYPOPHYSECTOMIZED RATS IN HYPOXIA[1]

By

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[1] This work was performed under the auspices of the United States Atomic Energy Commission.

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Abstract

The intraerythrocytic concentration of 2,3-DPG and the serum erythropoietin levels increase in weanling male, adult male, and female rats exposed to hypoxia for 5 hours. The serum titer of erythropoietin increases in male rats 3 hours after a 2-hour hypoxic exposure, but increases in 2,3-DPG do not occur. Increases in 2,3-DPG occur in 24-hour post-nephrectomized rats exposed to hypoxia, but the serum titer of erythropoietin is not increased. The level of 2,3-DPG and serum erythropoietin is not increased in male hypophysectomized rats exposed to a simulated altitude of 22,000 feet for 5 hours.

It is well known that the oxygen dissociation curve of hemoglobin measured *in vitro* shifts to the right following exposure to hypoxic conditions, and it has been assumed,[1,2] and recently demonstrated,[3] that this shift significantly increases the amount of oxygen delivered to the tissues. Eaton *et al.*[4] have noted that *in vivo* the hemoglobin of the circulating red cells does not come to equilibrium with the oxygen tension of the tissues, and have emphasized the importance of the oxygen affinity of hemoglobin within the red cell in supplying oxygen to the tissues. It has become increasingly clear during the last few years that the oxygen affinity of hemoglobin depends primarily upon the intraerythrocytic concentration of organic phosphate compounds,[5,6] particularly 2,3-diphosphoglycerate (2,3-DPG): red cells with high concentrations of 2,3-DPG have a decreased affinity for oxygen.

The supply of oxygen to the tissues in animals adapting to hypoxia can also be improved by increasing the circulating red cell mass through the production of new red cells by the action of erythropoietin. Significant elevations of serum erythropoietin occur soon after exposure to hypoxic conditions, although increases in red cell mass resulting from hormonal action require more time due to the maturation time of erythroid cells within the hematopoietic tissues. In this paper the interrelationships between changes in the concentration of red cell 2,3-DPG and circulating levels of serum erythropoietin will be explored in experimental situations known to alter erythropoietin production.

Materials and methods

All experiments were performed with Sprague-Dawley rats. The adult females weighed 310 ± 20 Gm. and the males 345 ± 40 Gm. Weanling male rats 22 days of age weighed 50 ± 8 Gm. Male rats hypophysectomized by Horton Laboratories, using the parapharyngeal approach, were weighed for 3 weeks, and only those

showing growth stasis at 165 ± 15 Gm. were used.

Normal male and female rats, hypophysectomized male rats, and 24-hour post-nephrectomized male rats were exposed to a simulated altitude of 22,000 feet in a decompression chamber. Unless otherwise stated, the rats were bled from the dorsal aorta under ether anesthesia immediately after a 5-hour hypoxic exposure. In one case one group of rats was bled immediately after a 2-hour hypoxic exposure, and another group was bled 3 hours after the end of a 2-hour hypoxic exposure.

Determinations of hematocrit, hemoglobin, and 2,3-DPG were performed on aliquots of heparinized blood immediately after blood sampling. Hemoglobin was determined using the cyanmethemoglobin technique. Erythrocytic 2,3-DPG concentrations were determined using the ultraviolet method described in the Sigma Chemical Company Technical Bulletin No. 35-UV. Serum erythropoietin levels were bioassayed in the 7-day post CO female LAF_1/JAX plethoric mouse as previously described.[7] One milliliter of serum was injected subcutaneously. The results are expressed as the 72-hour incorporation of ^{59}Fe into the calculated blood volume, which was assumed to be 7 per cent of the body weight.

Male rats were nephrectomized as previously described.[7] In one group of 24-hour post-nephrectomized male rats 1.5 cc. of blood/100 Gm. body weight was taken by cardiac puncture immediately before the hypoxic exposure. Two milliliters of blood were taken by cardiac puncture from one group of hypophysectomized rats, and these rats were exposed to hypoxia 25 days later.

The 2,3-DPG values are given as the mean ± 1 S.D. = $[\ (dev)^2/n\text{-}1\ ]^{1/2}$. The mole ratio 2,3-DPG: hemoglobin was calculated assuming a molecular weight of hemoglobin of 64,400. The underlined means are compared to the control means, and have a $P < 0.001$ as calculated by the Fisher t-test. Similar statistical values for comparisons between other experimental groups are given when relevant.

Results

Effect of hypoxia on 2,3-DPG concentrations in normal adult and weanling rat erythrocytes. The mole ratio of 2,3-DPG hemoglobin of normal rats as shown in Table I is not significantly different, although there is a suggestion that the μmoles of 2,3-DPG/Gm. hemoglobin is slightly higher in male rats compared to female rats. The serum erythropoietin titer is undetectable in adult rats and barely, but significantly, detectable in the male weanling rat.

All normal animals (Groups 2,4 and 8) respond to 5 hours hypoxia with a substantial increase in erythrocytic 2,3-DPG levels on all parameters tested and with marked increases in serum erythropoietin. The mole ratio of 2,3-DPG: hemoglobin increases 15 per cent in the female, 25 per cent in the adult male, and 33 per cent in the weanling rat. Male rats increased their serum erythropoietin levels significantly more than females.

A small, but significant, increase in serum erythropoietin levels occurs in male rats exposed to hypoxia for only 2 hours, whereas only a slight increase in 2,3-DPG levels, of doubtful significance, occurs. Three hours after the 2-hour hypoxic exposure the serum erythropoietin levels are markedly elevated, but the 2,3-DPG levels in all parameters are indistinguishable from the non-hypoxic controls.

Effect of hypoxia on 2,3-DPG concentrations in anephric and hypophysectomized rats erythrocytes. The 24-hour post-nephrectomized male rat (Group 2), as shown in Table II, has significantly lower concentrations of 2,3-DPG on all parameters compared to the normal male (Group 1). In contrast, hypophysectomized males (Group 5) have normal 2,3-DPG levels. Serum erythropoietin is undetectable in all of these rats.

Following a hypoxic exposure, the 2,3-DPG levels of 24-hour post-nephrectomy rats are significantly higher than in non-hypoxic anephric rats, yet the serum

erythropoietin levels are not elevated. The 2,3-DPG: hemoglobin ratio increased by about 58 per cent in the anephric rat and by about 55 per cent in the bled anephric rat, but a significant elevation in serum erythropoietin was seen in the bled anephric rat.

Hypophysectomized male rats do not elevate their 2,3-DPG or serum erythropoietin levels following a hypoxic exposure (Group 5 compared to Group 6) even when made anemic by bleeding (Group 7).

Discussion

Lenfant and co-workers,[8] and Gerlach et al,[9] have previously shown that the 2,3-DPG levels of red cells in humans and in rats are elevated in hypoxia within 5-6 hours. Our data corroborate these findings. The increase in 2,3-DPG levels, as well as the increase in serum erythropoietin, is greater ($P < 0.001$) in male rats than in female rats exposed to hypoxia for 5 hours. We have previously noted[7] this difference in erythropoietin production between male and female rats, and Wang and Fried[10] have reported similar results. Some correlation exists between the magnitude of increase in 2,3-DPG and serum erythropoietin levels in normal animals in these acute hypoxic exposures.

The 24-hour post-nephrectomized rat, whose 2,3-DPG levels are lower than the normal rat ($P < 0.001$) increases these values following 5 hours hypoxia to the level observed in normal rats exposed to the same hypoxia, but erythropoietin production does not occur. In contrast, the anemic 24-hour post-nephrectomized rat, with similar increases in 2,3-DPG levels, shows increased erythropoietin production. The 2,3-DPG levels of rats measured 3 hours after a 2-hour hypoxic exposure are not elevated above control levels, yet high levels of serum erythropoietin occur. It is clear, therefore, that increases in 2,3-DPG can occur without detectable changes in erythropoietin production; and, conversely, changes in erythropoietin production can occur without detectable increases in 2,3-DPG.

We have recently provided evidence[11] that the increased erythropoietin levels observed following a brief hypoxic exposure is the result of de novo synthesis. Since the 2,3-DPG levels of rats measured 3 hours after a 2-hour hypoxic exposure are not elevated, whereas increased blood levels of erythropoietin occur, the tissues involved in the biogenesis of erythropoietin are apparently more sensitive to hypoxic conditions than the processes involved in elevating the 2,3-DPG levels.

The ability of rats to respond to hypoxia with erythropoietin production progressively decreases after nephrectomy,[7,12] and by 24 hours erythropoietin production no longer occurs in rats exposed to 22,000 feet for 5 hours, although exposure to these hypoxic conditions immediately after nephrectomy does result in erythropoietin production. It is evident in the present experiments that erythropoietin production can occur in 24-hour post-nephrectomized rats if the animals are made anemic before the hypoxic exposure. We have found that a number of other experimental manipulations, such as increasing the degree of hypoxia, injection of bicarbonate, or the injection of red blood cell homogenates will promote extrarenal erythropoietin production in these anephric rats. These findings will be presented elsewhere. Of some importance is our consistent observation that the anephric rat has a greater hypoxic tolerance than the normal rat.[7] The explanation of this increased hypoxic tolerance is unknown, but we feel, on the basis of some preliminary observations, that the developing acidosis in the anephric rat is involved with a shift in the hemoglobin dissociation curve to the right. The decreased 2,3-DPG levels in the erythrocytes of anephric rats would be consistent with acidosis, since it has been clearly demonstrated in vivo[9,13-15] that 2,3-DPG levels are decreased when the blood pH is decreased. It is evident, however, that the anephric rat responds rapidly to hypoxia with marked increases in 2,3-DPG, probably as a result of alkalosis due to hyperventilation.

We have observed erythropoietin production in hypophysectomized rats exposed to altitudes of 28,000 feet, and conclude that the failure of erythropoietin formation to occur at 22,000 feet is because the supply of oxygen to the tissues at this lower altitude is adequate to supply their metabolic demands. The failure of hypophysectomized rats to increase their 2,3-DPG at 22,000 feet is, however, somewhat surprising. It has been suggested[16] that one mechanism for increasing the 2,3-DPG of red cells is based on the different affinity of 2,3-DPG for reduced and oxygenated hemoglobin. In hypoxia the arterial oxygen saturation decreases, free 2,3-DPG combines with the reduced hemoglobin, and more free 2,3-DPG is produced by the intraerythrocytic glycolytic enzymes. It appears reasonable to assume that the oxygen saturation of the arterial blood of the hypoxic hypophysectomized rat is reduced, and an increase in 2,3-DPG would be expected. Such an increase did not occur. We conclude, in agreement with Brewer et al.[17] that some unknown mechanism other than the differential binding of 2,3-DPG to reduced and oxygenated hemoglobin is involved in increasing intraerythrocytic 2,3-DPG levels in hypoxia. Studies of 2,3-DPG changes in the hypoxic hypophysectomized rat receiving various hormones may provide some insight into the mechanism of the 2,3-DPG increase.

Many investigators have determined the changes in serum erythropoietin levels in a number of species exposed chronically to different hypoxic conditions. These studies, as well as some recent observations, have been discussed most recently by Albrecht and Littell.[18] Peak increases in erythropoietin occur, depending upon the degree of hypoxia, only 1 or 2 days after the beginning of the hypoxic exposure, and the hormone decreases to undetectable levels during the next week even though increased erythropoietic activity is evident. Siri et al.[19] suggested that this decrease in erythropoietin production resulted from a decrease in functional hypoxia. We suggest that

one contributor to this decrease in functional hypoxia is the increase in the 2,3-DPG content of erythrocytes allowing a more efficient presentation of oxygen to the tissues. Data supporting this suggestion will be presented elsewhere.

References

1. Benesch R and Benesch RE: Intracellular organic phosphates as regulators of oxygen release by haemoglobin. Nature 221: 618-622, 1969.
2. Beutler E: "A shift to the left" or "a shift to the right" in the regulation of erythropoiesis. Blood 33: 496-500, 1969.
3. Oski FA, Marshall BE, Cohen PT, Sugerman HJ, and Miller LD: Exercise with anemia. The role of the left-shifted or right-shifted oxygen-hemoglobin equilibrium curve. Ann Intern Med 74: 44-46, 1971.
4. Eaton JW, Brewer GJ, and Grover RF: Role of red cell 2,3-diphospho-glycerate in the adaptation of man to altitude. J. Lab Clin Med 73:603 609, 1969.
5. Benesch R and Benesch RE: The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. Biochem Biophys Res Comm 26: 162-167, 1967.
6. Chanutin A and Curnish RR: Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch Biochem Biophys 121: 96-102, 1967.
7. Schooley JC and Mahlmann LJ: Erythropoietin production in the anephric rat. I. Relationship between nephrectomy, time of hypoxic exposure, and erythropoietin production. Blood 39: 31-38, 1972.
8. Lenfant C, Torrance J, English E, Finch CA, Reynafarje C, Ramos J, and Faura J: Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels. J Clin Invest 47: 2652-2656, 1968.
9. Gerlach E, Duhn J, and Deuticke B: Metabolism of 2,3-diphosphoglycerate in red blood cells under various experimental conditions. In: Brewer GJ, Editor: Red cell metabolism and function, New York, 1970, Plenum Press, pp. 155-174.

10. Wang F and Fried W: Renal and extrarenal erythropoietin production in male and female rats of various ages. J Lab Clin Med 79: 181-186, 1972.

11. Schooley JC and Mahlmann LJ: Evidence for the de novo synthesis of erythropoietin in hypoxic rats. Blood. In press.

12. Peschle C, Sasso GF, Rossanigo F, Mastroberardino G, and Lipparine, F: Ricerca sulla produzione extrarenale dell'eritropoietina indagini preliminari su animali binefrectomizzati e sottoposti ad ipossia. Riv Med Aeron e Sp 34: 24-31, 1971.

13. Messier AA and Schaefer KE: The effect of chronic hypercapnia on oxygen affinity and 2,3-diphosphoglycerate. Resp Physiol 12: 291-296, 1971.

14. Astrup P: Dependence of oxyhemoglobin dissociation and intra-erythrocytic 2,3-DPG on acid-base status of blood. II. Clinical and experimental studies. In: Brewer GJ, Editor: Red cell metabolism and function, New York, 1970, Plenum Press, pp. 67-79.

15. Bellingham AJ, Detter JC, and Lenfant C: Regulatory mechanisms of hemoglobin oxygen affinity in acidosis and alkalosis. J Clin Invest 50: 700-706, 1971.

16. Eaton JW, Brewer GJ, Schultz JS, and Sing CF: Variation in 2,3-diphosphoglycerate and ATP levels in human erythrocytes and effects on oxygen transport. In: Brewer GJ, Editor: Red cell metabolism and function, New York, 1970, Plenum Press, pp. 21-38.

17. Brewer GJ, Eaton JW, Weil JV, and Grover RF: Studies of red cell glycolysis and interactions with carbon monoxide, smoking and altitude. In: Brewer GJ, Editor: Red cell metabolism and function, New York, 1970, Plenum Press, pp. 95-114.

18. Abbrecht PH and Littell JK: Plasma erythropoietin in men and mice during acclimatization to different altitudes. J Appl Physiol 32: 54-58, 1972.

19. Siri WE, Van Dyke DC, Winchell HS, Pollycove M, Parker HG, and Cleveland AS: Early erythropoietin, blood, and physiological responses to severe hypoxia in man. J Appl Physiol 21: 73-80, 1966.

Table I. Effects of hypoxia on 2,3-DPG concentrations in normal adult and weanling rats

Subjects	No. of rats studied	Erythropoietin 72-hour ^{59}Fe incorporation *	Mole ratio 2,3-DPG/ hemoglobin	2,3-DPG ** µmoles/Gm. hemoglobin	2,3-DPG ** µmoles/ml. whole blood	2,3-DPG ** µmoles/ml. packed cells	Hemoglobin ** Gm./100 ml.	Hematocrit ** %
Female:								
1. Normal (control)	6	0.64 ± 0.23	1.4	21.2 ± 0.98	3.3 ± 0.19	7.1 ± 0.38	15.4 ± 0.64	46 ± 1.8
2. Normal + *** 5 hour HA	6	11 ± 0.55	1.6	24.5 ± 1.01	3.9 ± 0.12	8.6 ± 0.36	16.0 ± 0.61	45 ± 2.3
Male:								
3. Normal (control)	8	0.54 ± 0.09	1.5	23.5 ± 1.82	3.6 ± 0.33	8.0 ± 0.56	15.5 ± 0.63	45 ± 1.4
4. Normal + 5 hour HA	6	27 ± 0.65	1.9	29.3 ± 1.57	4.3 ± 0.31	10.0 ± 0.33	14.6 ± 0.78	43 ± 2.2
5. Normal + 2 hour HA	6	3.0 ± 0.18	1.7	26.2 ± 2.30	4.0 ± 0.39	9.2 ± 0.72	15.3 ± 1.10	44 ± 3.4
6. Normal + 2 hour HA + 3 hour later sacrificed	6	12.0 ± 0.32	1.4	23.2 ± 2.79	3.8 ± 0.56	8.5 ± 1.0	16.2 ± 0.75	44 ± 2.8
Weanling male:								
7. Normal	8	1.0 ± 0.12	1.4	22.1 ± 2.31	2.6 ± 0.10	7.6 ± 0.55	11.8 ± 0.94	34 ± 1.9
8. Normal + 5 hour HA	8	24 ± 1.1	1.9	29.3 ± 1.85	3.2 ± 0.27	9.7 ± 0.76	11.0 ± 0.96	33 ± 2.2

*
** Mean ± S.E.M.
*** Mean ± 1 S.D.
HA = 22,000 ft. The underlined means are compared to the control mean and the $P < .001$. Double underlined value $P < .005$.

Group No.	µmole/Gm. Hemoglobin t	µmole/Gm. Hemoglobin P	µmoles/ml. Whole Blood t	µmoles/ml. Whole Blood P	µmoles/ml. Packed Cells t	µmoles/ml. Packed Cells P	Hemoglobin t	Hemoglobin P	Hematocrit t	Hematocrit P
1 and 3	2.77	.02-.01	2.49	> .025	3.41	.005	-	-	-	-
3 and 7	1.35	-	8.47	< .001	1.47	-	9.26 <	.001	13.40	< .001
4 and 8	0.15	-	6.62	< .001	0.64	-	7.49 <	.001	8.07	< .001
3 and 5	-2.48	.05.-.025	1.92	.10-.05	-3.31	.01-.005	0.43	-	1.06	-

Table II. Effect of hypoxia on 2,3-DPG concentrations in anephric and hypophysectomized rats

Subjects	No. of rats studied	Erythropoietin* 72-hour ^{59}Fe incorporation	Mole ratio 2,3-DPG/ hemoglobin	2,3-DPG** µmoles/Gm. hemoglobin	2,3-DPG** µmoles/ml. whole blood	2,3-DPG** µmoles/ml. packed cells	Hemoglobin** Gm./100 ml.	Hematocrit** %
Male:								
1. Normal	8	0.54 ± 0.09	1.5	23.5 ± 1.82	3.6 ± 0.33	8.0 ± 0.56	15.5 ± 0.63	45 ± 1.4
2. 24-hour post-nephrectomy (control)	7	0.62 ± 0.18	1.1	17.8 ± 2.61	2.4 ± 0.32	6.2 ± 0.91	13.7 ± 0.49	40 ± 2.1
3. 24-hour post-nephrectomy + 5 hour HA***	7	0.67 ± 0.07	1.8	28.0 ± 1.86	3.9 ± 0.27	10.2 ± 0.81	13.9 ± 0.70	38 ± 1.5
4. Nephrectomy + 24 hour later bled + 5 hour HA	4	2.4 ± 0.49	1.8	27.4 ± 2.97	3.3 ± 0.49	9.9 ± 1.2	12.0 ± 1.10	33 ± 1.4
5. Hypophysectomy (control)	7	0.39 ± 0.07	1.4	22.4 ± 0.67	3.7 ± 0.18	7.6 ± 0.33	16.4 ± 0.88	48 ± 1.3
6. Hypophysectomy + 5 hour HA	7	0.67 ± 0.06	1.4	20.9 ± 1.45	3.7 ± 0.36	7.4 ± 0.65	17.7 ± 0.99	50 ± 3.7
7. Hypophysectomy + bled + 25 days later 5 hour HA	6	0.46 ± 0.12	1.3	20.7 ± 1.89	2.6 ± 0.17	7.6 ± 0.69	12.6 ± 0.89	34 ± 2.5

*
** Mean ± S.E.M
*** Mean ± 1 S.D.
HA = 22,000 ft. The underlined values are compared to the control mean, and the $P < .001$. Double underlined values $P \not> .01$

Group No.	µmole/Gm. Hemoglobin		µmoles/ml. Whole blood		µmoles/ml. Packed cells		Hemoglobin		Hematocrit	
	t	P	t	P	t	P	t	P	t	P
1 and 2	4.95	< .001	7.03	< .001	4.85	< .001	6.10	< .001	6.23	< .001
1 and 3	-4.72	< .001	1.59	-	-6.11	< .001	4.69	< .001	9.65	< .001
1 and 4	-2.85	.02-.01	1.48	-	-3.80	.005-.001	7.15	< .001	14.21	< .001
1 and 5	1.50	-	0.45	-	1.84	-	-2.30	.05-.025	-4.35	< .001
3 and 4	0.42	-	2.70	.025-.02	0.59	-	3.57	.01-.005	5.23	< .001

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mb per me.

June 7, 1972

Loretta Lizama
Technical Information Division
Bldg. 90, Room 1046

Dear Loretta

I am enclosing a copy of a manuscript entitled: "Erythropoietin Production and Erythrocytic 2,3-Diphosphoglycerate Changes in Normal, Nephrectomized and Hypophysectomized Rats in Hypoxia" by John C. Schooley and Lynn J. Mahlmann that has been submitted to The Journal of Laboratory and Clinical Medicine.

Sincerely,

Grace

Grace Walpole
Biology and Medicine Division
Bldg. 74

Enclosure (1)