NEURO-HUMORAL LINKS IN THE REGULATION OF ERYTHROPOIESIS

(UDC 616.155.194-009-092.9-07 : 616.155.1-007.1 + 616.155.1-007.1-02 : 612.275)

Yu. P. Baldin

Department of Pathological Physiology (Head, Honored Scientist of the Bashkir ASSR Professor V. A. Samtsov), Bashkir Medical Institute, Ufa
(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 7, pp. 21-27, July, 1965
Original article submitted February 14, 1964

No information could be found in the literature on the regulatory mechanisms of erythropoiesis in cases of "neurogenic" anemia caused by denervation of the carotid sinus reflexogenic zones in the process of acclimatization to hypoxia. We have therefore investigated the degree to which a lowered partial pressure of oxygen can modify the process of erythropoiesis and restore the normal composition of the red blood in animals with experimental "neurogenic" anemia caused by denervation of the carotid sinuses.

EXPERIMENTAL METHOD

Experiments were conducted on 71 male rabbits. The operation of denervation of the carotid sinuses was performed by the usual method under ether anesthesia [8].

The animals were "raised" daily in a pressure chamber at the rate of 16-17 m/sec to the equivalent of an altitude of 7500 m (total pressure 290 mm Hg), for an exposure period of 40 min on the first 2 days and 60 min on the first 2 days and 60 min on subsequent days.

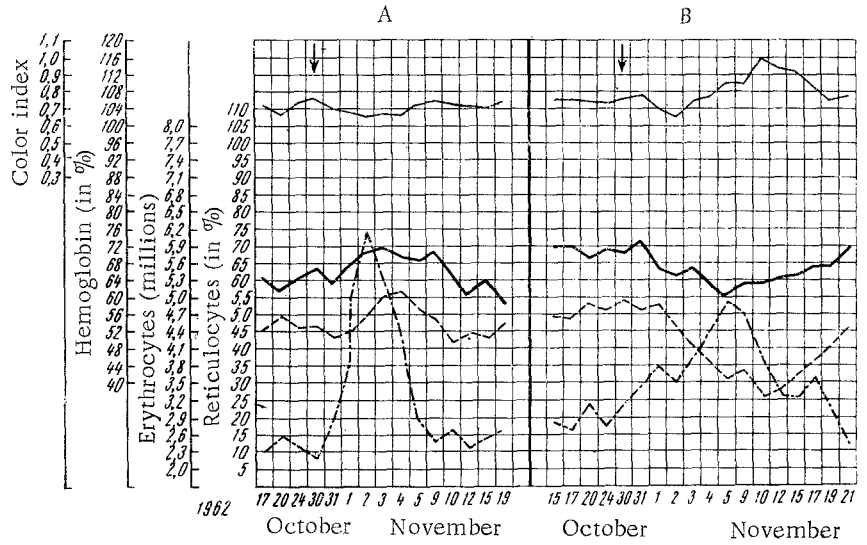
The blood was examined both immediately after the rabbits had been exposed in the pressure chamber and after an interval of 24 h. The bone marrow was extracted by puncture of the tibial epiphysis. Blood for the preparation of serum was obtained by puncture of the heart. The serum obtained (10-12 ml) was injected intravenously into healthy recipient rabbits. Peritoneal dialysis was carried out on the nephrectomized animals by the method developed by S. M. Shenderov [7], working in the laboratory directed by V. V. Parin. The serum bilirubin was determined by Endraskin's method.

EXPERIMENTAL RESULTS

On the second day after the operation of denervation of the carotid sinuses, eight rabbits were exposed to the action of a lowered partial pressure of oxygen. The development of anemia was not observed in these animals. In five control rabbits undergoing the operation but not "raised to a high altitude" anemia developed and reached its maximum on the 14th-16th day (when the erythrocyte count fell to 1,260,000-1,440,000 and the Hb concentration to 17-19% of its initial level, while the reticuloctyte count was more than doubled). In the experimental animals exposed to the action of hypoxia during the first 6-8 days, the erythrocyte count did not fall below the initial level, but the color index fell slightly and the reticulocyte count rose by 300-500%. On the 14th-16th day the Hb concentration of these rabbits was increased by 4-9% and the erythrocyte count by 410,000-850,000. Analysis of the partial erythrograms showed that the number of 3rd order erythroblasts had increased by 4.5-8.5%, whereas in the control animals undergoing the operation the increase was not more than 2%.

It was previously shown [1, 3] that the anemia developing after denervation of the carotid sinuses is due to stimulation of the spleen, which under these circumstances secretes active hemolytic substances capable of causing the development of anemia in recipient animals also.

It is clear from the figure (B) that injection of serum obtained from a control animal, subjected to the operation but not exposed to hypoxia, during the period of marked anemia led to the development of severe anemia in the recipient.



Changes in the number of erythrocytes (----), the Hb concentration (———), the reticulocyte count (-.-.-.), and the color index (———) in rabbits in response to injection of 10 ml of serum (↓) of a donor rabbit take on the 14th day after denervation of the carotid sinuses and subsequent daily acclimatization to hypoxia (A) and in response to injection of serum taken on the 14th after denervation of the carotid sinuses (B).

On the other hand, the serum of the rabbits "raised" in the pressure chamber from the second day after denervation of the carotid sinuses and taken from them on the 14th-16th day, i.e., at the same time as from the control rabbits undergoing the operation, possessed marked erythropoietic activity (see figure, A). In all six recipient rabbits, after injection of this "hypoxic" serum, the erythrocyte count was increased on the 3rd-4th day by 700,000-1,200,000, the Hb concentration was raised by 6-10%, and the reticulocyte count also showed a sharp increase.

Hence, by keeping animals in conditions of hypoxia, the development of anemia after denervation of the carotid sinuses can be prevented; the serum of such animals possesses a marked erythropoietic effect, due to the presence of hemopoietic substances (erythropoietins). This conclusion is in agreement with the views of those investigators who have reported that oxygen lack has a stimulant effect on the formation of erythropoietins [10, 14, 15]. At the same time, exclusion of the carotid sinuses in animals in conditions of a lowered partial pressure of oxygen is known to lead to a more profound degree of hypoxemia than in healthy, intact animals [2].

If we accept the view that erythropoietins are produced by the spleen [11], the blood serum of animal undergoing splenectomy and subsequently exposed to the action of hypoxia should not posses erythropoietic properties. However, our experiments showed that the serum of splenectomized animals kept in hypoxic conditions for the same periods as intact rabbits caused a marked increase in all the red blood indices (see table). We therefore conclude, like certain other investigators [6, 14], that the spleen is not the principal site of formation of erythropoietins.

Nephrectomy was performed simultaneously on the second group of experimental rabbits. On the day after the operation three animals were "raised to a high altitude" daily for 7 days and another three rabbits served as the control group. In the animals exposed to the action of hypoxia, a marked redistributive erythrocytosis was observed immediately after the stay "at a high altitude." However, examination 24 h after each "ascent" revealed a progressive lowering of the erythrocyte count in their blood and of their hemoglobin concentration. After seven "ascents" the erythrocyte count was lowered by 890,000-1,310,000 and the reticulocyte count was raised from 19-25 to 34-50 ‰. Examination of bone narrow puncture material during this period showed a change in the relative proportions of the leukoblastic and erythroblastic cells, in the direction of an increase in the myeloid series. At the same time there was no slowing of maturation of the cells of the erythroid series, for counts of the partial erythrograms revealed an increase in the number of most mature forms, i.e., of the erythroblasts of the 2nd and 3rd orders. The serum bilirubin concentration rose from 0.2 to 0.50-0.62 mg %.

In the three control animals which were not "raised to a high altitude" the erythrocyte count on the 7th day

Changes in Erythrocyte Count, Hb Concentration, and Reticulocyte Count in Recipient Rabbits in Response to Injection of Serum of Nephrectomized, Splenectomized Rabbits Kept in Hypoxic Conditions, and of Nephrectomized Rabbits Kept in Normal Conditions

Characteristics of donor	Serial No. of recipient rabbit	Before injection of serum				After injection of serum				
		erythrocytes (millions)	Hb (%)	color index	reticulocytes (‰)	erythrocytes (millions)	Hb (%)	color index	reticulocytes (‰)	day of investigation
Splenectomized rabbits kept in hypoxic conditions	9	5,13	73	0,71	17	5,76	76	0,66	53	3rd
	10	4,98	68	0,69	21	5,93	78	0,66	44	4th
	11	5,0	76	0,76	15	6,58	85	0,65	46	4th
	12	4,66	71	0,77	24	5,99	80	0,67	61	5th
	13	4,89	70	0,72	19	5,67	77	0,68	45	4th
	14	5,22	82	0,78	14	6,13	89	0,72	36	4th
	15	4,73	68	0,72	23	5,21	73	0,7	39	5th
	16	4,67	65	0,7	18	5,14	69	0,67	27	3rd
Mean		4,91	71,6	0,74	18,8	5,80	78,3	0,67	43,8	
		M_{diff}		$\pm m$ σ t P		0,89±0,13 0,39 6,8 <0,001	6,7±0,8 2,5 8 <0,001	−0,05±0,01 0,03 5 <0,01	24±3,5 10 6,8 <0,01	
Nephrectomized rabbits kept in hypoxic conditions	24	4,4	64	0,72	18	4,71	66	0,70	37	3rd
	25	4,96	68	0,69	16	4,73	62	0,65	26	3rd
	26	5,71	74	0,64	13	6,17	79	0,64	32	4th
	27	5,06	68	0,68	16	5,01	67	0,67	14	3rd
	28	4,88	66	0,68	22	5,17	69	0,67	35	4th
	29	5,26	72	0,69	14	5,58	74	0,67	25	4th

	30 31	4,80 4,63	67 64	0,69 0,69	19 21	4,65 4,52	63 60	0,68 0,66	27 30	4th 3rd
Mean		4,96	67,8	0,68	17,3	5,06	67,5	0,66	28,2	
		M_{diff}		$\pm m$ σ t P		0,1±0,09 0,26 1 >0,3	—0,3±0,8 4 0,3 >0,7	—0,01±0,003 0,01 3 <0,02	10,8±2,3 6,7 4,6 <0,01	
Nephrectomized rabbits kept in normal conditions	24a 25a 26a	4,47 4,89 5,33	63 66 70	0,71 0,68 0,66	27 21 15	4,32 4,96 5,28	61 67 66	0,70 0,68 0,63	31 14 17	3rd 3rd 3rd
Nephrectomized rabbits kept in normal conditions	27a 28a 29a 30a 31a	4,99 4,77 4,91 4,84 4,58	67 66 68 65 67	0,68 0,7 0,69 0,67 0,74	19 16 21 16 18	4,69 4,99 4,99 4,42 4,35	62 64 68 52 63	0,67 0,65 0,69 0,59 0,73	11 19 22 10 23	3rd 3rd 3rd 4th 3rd
Mean		4,84	66,4	0,69	19,1	4,75	62,8	0,66	18,3	
		M_{diff}		$\pm m$ σ t P		—0,09±0,06 0,18 1,5 =0,2	—3±1,4 4 2 >0,05	—0,02±0,007 0,02 2,8 <0,05	—0,7±1,8 5,3 0,3 >0,7	

after nephrectomy was lowered by 670,000-860,000 and the reticulocyte count was lowered by more than 50% by comparison with the original values. Myeloid elements predominated in the bone marrow and signs of severe depression of erythropoietic activity were present. These signs included the almost complete absence of polychromatophilic and orthochromic erythroblasts. The serum bilirubin was raised to the same degree as in the experimental animals (0.44-0.56 mg %).

Injection of the blood serum obtained on the 8th day after nephrectomy from the animals exposed to the action of hypoxia and from the control animals produced different hematological effects in the healthy recipient rabbits (see table).

A statistically significant increase in the reticulocyte count in the blood was observed in all the recipients with the exception of the two groups of animals injected with blood serum from nephrectomized rabbits not exposed to the action of hypoxia, and with the serum of healthy animals. However, a statistically significant increase in the erythrocyte count was observed in the recipient rabbits only after receiving injections of serum from intact and splenectomized animals kept in hypoxic conditions.

In view of the fact that the blood serum of the intact and the splenectomized "hypoxic" animals possesses marked erythropoietic activity, whereas nephrectomy leads to a decrease in its erythropoietic activity, our investigations confirm the results obtained by other authors [9, 12, 13], who report the great importance of the kidneys in the production of erythropoietins.

In addition, attention should also be drawn to the fact that the blood serum of the nephrectomized animals kept in hypoxic conditions possesses a marked reticulocytogenic effect which, as the results showed, is independent of the retention of metabolic products in the body of the nephrectomized animals. Evidently the conditions of hypoxia activate and accelerate the maturation of the erythroblastic elements of the bone marrow in nephrectomized rabbits by means of the formation of active humoral substances. This is confirmed by the fact that administration of the "hypoxic" serum of such animals causes proliferation of the bone marrow cells and an increase in the number of reticulocytes in the peripheral blood of the recipients.

It is difficult to explain the mechanism of the reticulocytogenic effect of this serum. Hypotheses have recently been put forward concerning the excistence of two erythropoietic factors—a thermostable and a thermolabile factor [13]. In the opinion of the authors of this hypothesis the first accelerates division of the bone marrow cells while the second takes part in the synthesis of the hemoglobin molecule. These authors suggest that the thermolabile factor is produced in the kidneys, but the site of formation of the thermostable factor is not known.

Our results could be explained on the basis of this concept which, although very debatable, has already been accepted by some Soviet workers [5]. The conditions of hypoxia evidently cause the increased production in nephrectomized animals of only one of the erythropoietic factors, that stimulating proliferation of the bone marrow cells, for the producers of the second factor concerned in the synthesis of hemoglobin (the kidneys) are absent. Hence, the blood serum of such donors has an effect which is predominantly and almost entirely reticulocytogenic in character.

LITERATURE CITED

1. Yu. P. Baldin, The connection between the functional state of the receptors of the carotid reflexogenic zone and the systemic blood. Candidate dissertation, Chelyabinsk, (1962).
2. N. N. Beller, Byull. éksper. biol., 6, (1957), p. 12.
3. R. A. Durinyan, Izv. AN Armyansk. SSR, Biol. nauki, No. 5, (1960), p. 13.
4. L. I. Idel'son, Probl. gematol., No. 12, (1961), p. 11.
5. E. L. Kan, In book: Problems in Hematology and Anticoagulant Therapy [in Russian], Leningrad, (1960), p. 59.
6. N. A. Fedorov, M. G. Kakhetelidze, and I. K. Koryakina, Probl. gematol., No. 11, (1960), p. 17.
7. S. M. Shenderov, Byull. éksper. biol., No. 2, (1962), p. 125.
8. A. Ya. Yaroshevskii, Byull. éksper. biol., No. 11, (1950), p. 320.
9. A. Ya. Yaroshevskii, Endogenous Stimulators of Hemopoiesis (Erythropoietins) [in Russian], Moscow–Leningrad, (1963).
10. E. Bonsdorff, Acta physiol. scand., 15, (1948), p. 61.
11. P. Gley, Bull. Acad. nat. Med. (Paris), 136, (1952), p. 521.
12. Z. Kuratowska, B. Lewartowski, and E. Michalak, Blood, 18, (1961), p. 527.
13. J. W. A. Linman and F. H. Bethell, Factors Controlling Erythropoiesis. Springfield, (1960).

14. S. J. Piliero and P. T. Medici, Acta haemat. (Basel), 25, (1961), p. 220.
15. F. Stohlman, Jr., Pediatrics, 23, (1959), p. 385.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
