“Peroxidase Response” of Erythron in Anemia

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In a preceeding paper¹⁾ of mine, I reported that the peroxidase activity of erythrocytes was greatly altered in cases of anemia and that alteration in these cases consisted mainly in a striking increase of strongly peroxidase-positive erythrocytes as revealed by a counting chamber method of peroxidase reaction devised by myself.

In the present paper, I desire to report a certain correlation between the hemopoiesis and the peroxidase activity of erythrocytes, on the basis of the result of serial examination of six cases of various kind of anemia and to discuss the hematological significance of this method.

EXPERIMENTAL

Method

The routine hematological procedures and the new technique of the counting chamber method²⁾ devised by myself were used.

Materials

A brief record of each case will be given below:

Case 1. T. K., 1 year and 3 months old, female. She was born one month prematurely and bottle-fed. She had developed hypochromic anemia that seemed to respond to iron. But she was discharged before the anemia was completely recovered.

Case 2. H. K., 22 years old, female. Case of idiopathic thrombocytopenic purpura. She had often purpurul skin manifestation, and besides, at the time of menstruation, a large amount of blood loss causing a profound anemia, which was treated with multiple transfusion with improvement.

Case 3. E. K., a female newborn. She was the first child of healthy parents, and born twelve days before term, weighing 2360 g. Delivery

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was normal. On the third day of life, she developed jaundice which gradually increased thereafter. On the thirteenth day, suddenly she developed drowsiness and convulsions, and she was admitted to the University Hospital. For the following seven days these symptoms persisted, besides, she had fever, cyanosis, dyspnea occasionally simulating Cheyne-Stokes respiration and vomiting. At the onset, the examination of blood showed normal erythrocytes and hemoglobin level, though there was a macrocytosis, an increase of nucleated erythrocytes and an increase of icterus index. Anemia developed gradually within the following few weeks, and a splenomegaly ensued. Liver was palpable two finger breadth below the right costal margin on the day of admission. By an energetic and careful treatment, the patient improved and was discharged on the eightieth day of birth with sequelae of occasional mild attacks of convulsions resembling chorea minor and nodding spasm (suggesting kernikterus).

Subsequent tests of the blood groups of the members of the family showed that the father was of group O, Rh-positive, and the mother was of group AB, Rh-negative. And anti-Rh agglutinin was demonstrable in the mother's serum on the twentieth day after delivery. And the diagnosis of erythroblastosis fetalis due to Rh incompatibility was thus established.

Case 4. M. I., a 5 year 11 months old girl. She entered the Hospital because of bronchopneumonia. On admission, loud basal systolic murmurs and clubbed fingers, suggesting congenital heart malformation, were found. She was treated effectively treated with penicillin and discharged. In the next year she was readmitted because of meningeal signs and respiratory distressis. The diagnosis at this time was purulent meningitis, bronchopneumonia and glomerulonephritis. During the course of illness, massive intestinal hemorrhages occurred and moderate anemia developed. She was treated with penicillin, streptomycin and blood transfusions and improved.

Case 5. B. M., 36 year old man. A typical case of pernicious anemia showing a moderated, macrocytic anemia with megaloblastic bone marrow and achlorhydria and neurological symptoms, such as numbness and tingling of the hands and feet, for a few years. The course of illness was not influenced by iron treatment, but the patient responded well to vitamin B₁₂, while achlorhydria of gastric juice alone remained uninfluenced.

Case 6. J. K., 1 year old boy. He was admitted to the Hospital because of intermittent diarrhea, pallor and weakness. He was born at full term normally. During the pregnancy his mother was suffering from beriberi which was treated with thiamine. The weight at birth was about 3400 g. He was breast-fed, and for a few weeks prior to entry, a small amount of fruit juice had been given but no other food

up to the day of admission. He could not sit until the ninth month of age. There was no history of acute infections; and he had not been vaccinated yet. For about three months before admission, he had intermittent diarrhea and vomiting of milk, and he gradually developed pallor and weakness.

On admission, physical examination showed a moderately anemic, hypotrophic, and irritable infant weighing 8.25 kg. (normally 9.17 kg.) with enlarged liver as well as enlarged spleen (one and a half finger breadth below the costal margin). Cervical and inguinal lymph glands were enlarged. He had a slight ankle edema. There was no evidence of the involvement of the spinal cord and of visible mucous membrane. The acidity of gastric juice was within normal range. Blood showed a moderate macrocytic anemia with high color index and granulocytopenia with moderate hypersegmentation of nuclei of the neutrophils. Bone marrow was megaloblastic. After entry, he was fed on mother's milk, cow's milk and fruit juice; thiamine, vitamin C and glucose were given intravenously every day. During the first two weeks of hospitalization, prior to starting any specific treatment for megaloblastic anemia, a marked reticulocytosis occurred unexpectedly and a gradual increase of erythrocyte count and hemoglobin level and granulocyte count followed, and simultaneously megaloblasts disappeared in the bone marrow. There was an incidence of bronchopneumonia about the end of the third hospital week, when vitamin B₁₂ and folic acid were given for the purpose of preventing a possible recurrence of anemia and leucopenia, while penicillin also was given. The patient responded well and was discharged after recovery.

Results of Peroxidase Activity

Case 1 (Table I and Fig. 1). At first the strongly peroxidase-positive erythrocyte level was low in spite of the presence of a moderate hypochromic anemia, but it was temporally elevated by an iron medication.

Case 2 (Table I and Fig. 2). In this case a reciprocal relationship was seen between red cell count and hemoglobin value on one hand and the strongly peroxidase positive erythrocyte level on the other—quite an expected relation: when the former were low, the latter was high, and vice versa.

Case 3 (Table I and Fig. 3). In this case too, the reciprocal relationship between red cell count and hemoglobin value on one hand and the strongly peroxidase-positive erythrocyte level on the other was found. And an elevation of strongly peroxidase-positive erythrocyte level was found in a fair parallelism with that of reticulocyte level, suggesting a possible direct relation between a part of the strongly peroxidase-positive erythrocytes and reticulocytes.

TABLE I

Case no.	Name	Age	Sex	Date	Diagnosis	Hematological data				Peroxidase activity of erythrocytes		
						R.B.C. mill.	Hb g/dl	C.I.	Reticulocytes %	+	+	-
										%	%	%
1	T.K.	1y 3m	F	51-10-31	Iron deficiency anemia of bottle-fed infant	370	6.5	0.59	—	—	—	—
				"-11- 4		335	5.5	0.55	—	0	99.8	0.2
				"-11- 7		355	5.5	0.52	—	—	—	—
				"-11-13		380	6.1	0.54	—	21.4	77.6	1.0
				"-11-15		413	8.6	0.69	—	2.4	95.8	1.8
2	H.K.	22y	F	44- 1-11	Idiopathic thrombocytopenic purpura	400	11.7	0.98	—	11.0	98.8	0.2
				"- 1-25		140	4.8	1.14	db. 1.2	83.8	16.2	0
				"- 1-29		210	4.8	0.76	p.b. 0.4	77.8	22.2	0
				"- 2- 5		300	4.4	0.49	d.b. 0.9	38.0	61.2	0.8
				"- 2-24		450	11.0	0.81	p.b. 0.4	3.0	97.0	0
				"- 3- 8		450	14.5	1.07	d.b. 1.5	12.4	87.6	0
				"- 3-30		350	12.4	1.18	p.b. 1.1	1.0	98.0	1.0
				"- 5-30		410	11.5	0.93	d.b. 0.1	0.2	99.8	0
									p.b. 0			
3	E.K.	13 days	F	50- 3-13	Erythroblastosis fetalis due to Rh incompatibility	650	18.0	1.03	0.7	—	—	—
				"- 3-19		637	19.1	1.11	2.0	—	—	—
				"- 3-23		566	15.6	1.02	1.2	—	—	—
				"- 3-26		429	13.1	1.13	0.9	1.6	98.3	0.1
				"- 4- 3		370	9.0	0.61	5.3	31.0	68.4	0.6
				"- 4- 7		340	10.2	0.90	6.3	19.4	80.2	0.4
				"- 4-11		345	8.7	0.8	7.5	34.0	64.8	1.2
				"- 4-15		350	10.4	0.9	5.6	30.2	69.7	0.1
				"- 4-21		335	11.0	1.0	5.7	40.0	59.8	0.2
				"- 4-28		330	8.4	0.8	5.3	29.2	70.5	0.3
				"- 5- 7		415	—	—	3.1	28.0	72.0	0
				"- 5-11		460	11.9	0.8	2.6	10.0	89.8	0.2
4	M.I.	5y 11m	F	49- 7-11	Cong. heart disease	410	—	—	—	15.8	84.2	0
				"- 7-16		350	—	—	—	5.2	94.6	0.2
				50- 4-17	Acute nephritis	230	4.5	0.65	6.0	56.0	44.0	0
				"- 4-19		240	5.1	0.71	3.2	28.4	71.6	0
				"- 4-21	Intestinal bleeding	270	6.2	0.77	6.0	57.7	42.2	0.1
				"- 4-26		220	4.1	0.62	6.5	37.0	63.0	0
				"- 4-29		220	5.0	0.76	9.0	57.6	42.4	0
				"- 5- 2		300	5.8	0.64	2.4	31.0	69.0	0
				"- 5- 4		290	8.3	0.95	1.8	35.0	65.0	0
				"- 5- 7		290	7.5	0.86	0.4	67.0	33.0	0
				"- 5-12		350	6.6	0.63	—	13.6	86.4	0
				"- 5-18		300	4.7	0.52	4.6	21.0	79.0	0
				"- 5-26		310	—	—	10.0	0.8	99.0	0.2
				"- 5-31		—	6.5	—	—	—	—	—
				"- 6-13		310	10.5	1.13	1.4	0.1	99.9	0

Abbreviations: d. b. diffuse basophilia of erythrocyte.
 p. b. punctate basophilia of erythrocyte.
 R.B.C. Red blood corpuscles.
 C.I. Color index.

Case 4 (Table I and Fig. 4). This case showed rather eventful courses of erythrocyte count, hemoglobin value, reticulocyte count and strongly peroxidase-positive erythrocyte level, probably because of multiple complications of different clinical entities, and yet, a rough relationship between red cell count and hemoglobin value on one hand and strongly peroxidase-positive erythrocyte count on the other will be seen.

Case 5 (Tables II, III, and IV, Figs. 5, 6, 7 and 8). Neither clinical picture nor laboratory data including the peroxidase activity of erythrocytes were changed by iron treatment, but a great alteration was induced by vitamin B₁₂. At first, within five hours after the first intramuscular injection of 15 γ of this vitamin the peroxidase activity of megaloblasts in the sternal marrow was remarkably increased, which is shown in Table IV as an increase of peroxidase-

positive and even strongly peroxidase-positive cells of megaloblast series. This phenomenon was followed by a decrease of megaloblasts and an active proliferation of macroblasts, though the latter did not become evident until forty eight hours after the first vitamin B₁₂ injection. The high peroxidase level of erythroblasts persisted about on week, during which time erythropoietic process had been restored to the normal way, and developmental and proliferative activity was highly stimulated. A fortnight later on, the peroxidase activity of normoblasts were found gradually decreasing to the normal level. (At the same time a small number of extracellular nuclei with high peroxidase content were visible). The strongly peroxidase-positive type of erythrocytes also began to increase in the bone marrow at the same time as that of nucleated erythrocytes, and they, both strongly peroxidase-positive and nucleated erythrocytes decreased again in successive stage. In the circulating blood, also, the strongly peroxidase-positive erythrocytes began to increase in number soon after vitamin B₁₂ treatment was commenced and reached its

Fig. 1 (Case 1)

Fig. 2 (Case 2)

maximum on the seventh day of specific treatment, and then gradually decreased in number. As to the reticulocyte reaction it developed and reached its maximum on the fifth day of specific treatment and then subsided again. It was noted that the strongly peroxidase-positive erythrocyte count was always larger than the reticulocyte count, and that the high enzymic activity persisted more or less long after the reticulocytosis had disappeared. Erythrocytes and hemoglobin increased gradually but steadily and on the sixteenth day of the specific treatment they were 3.76 millions per cmm. and

11.3 g. per 100 cc. respectively. On the fourteenth and the twenty first day of the specific treatment, the peroxidase activity of bone marrow elements of granulocyte series were found to be much increased when the granulocytopoietic activity was also hyperactive, showing a decrease of erythroid-myeloid ratio in the bone marrow and an increase of granulocyte count in the peripheral blood.

Some of megakaryocytes in the bone marrow were peroxidase-positive or/and even strongly peroxidase-positive in the second and the third week of the specific treatment, during which period platelet count in the blood had been more or less getting higher.

Case 6 (Tables V and VI and Fig. 9). In this case, the peroxidase activity of erythrocyte was very high on the second day of admission, and a few days later a reticulocyte response developed without any specific treatment, and a gradual increase of erythrocyte count and hemoglobin level followed. One month later, after an incidence of bronchopneumonia, his general conditions and blood picture were found much improved, and at that time both the strongly peroxidase-positive erythrocyte count and the reticulocyte count were lower than before.

SUMMARY OF RESULTS AND DISCUSSION

The present paper reports the results of hematological investigations

esp. that of peroxidase activity by the new technique of counting chamber copper peroxidase reaction in six cases of anemia, i.e. one case of iron deficiency anemia of a weanling, one case of idiopathic thrombocytopenic purpura, one case of erythroblastosis fetalis due to Rh incompatibility, one case of anemia due to intestinal hemorrhage, one case of pernicious anemia and one infantile case of megaloblastic anemia. A certain regularity was noticed in the relationship between the peroxidase activity of erythrocyte and the hemopoietic activity of erythron. In Cases 2,3 and 4, during their erythrocyte counts and hemoglobin levels had been normal, and in deficiency anemia Cases 1 and 5, during the specific treatment had not been given, the peroxidase activity of erythrocytes was relatively low. But in all of the presented cases, the peroxidase activity of erythrocytes was greatly stimulated at the early stage of blood regeneration, and this was shown rather as a sign of blood regeneration. This phenomenon was found in a reciprocal relationship between the erythrocytes and hemoglobin level on one hand and the strongly peroxidasepositive erythrocyte count on the other in Cases 2,3 and 4; and it

Fig. 3 (Case 3)

Fig. 4 (Case 4)

TABLE II

Date 1951	R.B.C. million	Hb g/dl	C.I.	Erythro- blasts per 200 W.B.C.	Retic. %	Peroxidase activity of erythrocytes			Platelets (thousands)	Differential count						Neuclear shift of neutrophils								
										WBC	Eos.		Lym.		Mon.		Neut.		I	II	III	IV	V	VI
						+	+	-			%	absol. count	%	absol. count	%	absol. count	%	absol. count						
Aug. 20	172	6.2	1.20	3	1.6	5.0	94.2	0.8	28.3	4200	5.0	210	15.2	640	5.8	240	74.0	3110	11	39	48	2	0	0
28	277	7.5	0.90		0.6	14.0	85.6	0.4	47.0	4200	4.4	180	27.5	1160	5.6	240	62.5	2620	5	28	36	28	3	0
Sept 3	210	6.3	1.00	4	0.7	5.4	94.2	0.4		4000	2.0	80	38.0	1520	3.0	120	52.0	2080	5	39	43	9	4	0
7	206	7.2	1.17		0.6	18.4	81.4	0.2	74.0	5800	3.0	120	43.0	1720	5.0	200	47.0	1880	6	26	43	19	4	0
10	190	8.3	1.46	2		13.0	87.0	0		4000	4.5	260	40.0	2320	6.5	380	49.0	2840	8	20	40	23	7	2
12	220	7.5	1.14			3.0	94.0	3.0			1.8	70	35.2	1410	3.0	120	60.0	2400	3	18	42	30	4	3
10:15 a.m.	206	7.5	1.21			13.4	86.4	0.2	94.7	4100	3.0	120	51.0	2090	6.0	250	40.0	1640	2	18	30	44	2	4
3:15 p.m.						30.5	69.4	0.1																
10:15 a.m.						23.0	77.0	0																
10:15 a.m.	219	7.6	1.16	1	3.0	35.4	64.5	0	70.0	4000	4.0	160	45.0	1800	1.0	40	50.0	2000	1	21	42	28	6	2
10:15 a.m.	267	8.3	1.04		7.4	61.0	39.0	0	5400	5400	1.5	80	39.5	2130	10.5	570	48.5	2620	6	35	42	14	3	0
10:15 a.m.	315	9.2	0.97			65.0	35.0	0	4400	4400	4.0	180	36.0	1580	12.0	530	48.0	2110	2	15	61	14	3	0
10:15 a.m.	300	9.0	1.0		3.5	8.0	87.6	4.4	5400	5400	4.0	220	37.0	2000	11.0	590	48.0	2590	4	33	46	10	5	2
21	367	9.0	0.82		5.6	23.6	76.2	0.2	85.0	5900	2.0	120	38.0	2240	7.0	410	53.0	3130	6	28	36	22	6	2
23						31.4	67.6	1.0																
25																								
26	356	10.1	0.95		0.9	21.6	78.4	0	121.0	5600	3.9	220	30.6	1710	5.0	280	55.5	3110	4	22	44	26	6	0
28	376	11.3	1.00		6.9	12.4	87.6	0	7200	7200	4.5	320	28.0	2020	5.0	360	62.5	4500	5	28	40	20	5	2
30						22.0	78.0	0																
Oct. 2				2	1.0	37.4	62.6	0	7000	7000	5.5	385	34.5	2420	8.0	560	42.0	2940	5	22	38	31	2	2
3	10.8				1.4	7.6	92.4	0																
6					1.0	4.0	96.0	0																
8					1.5	0.4	98.8	0.8	98.5	6600	0	0	29.0	1910	6.5	430	64.5	4260	5	45	39	9	2	0
10					0.6	0.6	99.4	0																
12					1.3	4.2	95.6	0.2																
15					0.3	0.8	99.2	0	6400	6400	7.0	450	34.5	2210	5.5	350	53.0	3390	6	41	40	13	0	0
17	400	11.0	0.92		0.5	0.2	99.8	0																
19					1.1	0	98.6	1.4																
22					0.8	0	100	0	110.2	6600	3.0	200	31.5	2080	9.5	630	56.0	3700	7	40	38	13	2	0
24	418	11.7	0.93		1.1	0	100	0																
26					1.0	0	100	0																
27	402	11.3	0.94		1.0	0	100	0	7000	7000	4.0	280	38.0	2660	5.0	350	53.0	3710	8	34	43	13	2	0
31	401	11.5	0.96		0.7	0	100	0	96.0	7000	4.0	280	35.0	2450	5.0	350	56.0	3920	4	11	48	30	7	0
8	392	12.1	1.03		0.7	0	100	0	98.0	6800	3.5	240	37.0	2520	4.5	310	55.0	3740	2	14	44	36	4	0
Nov 14	403	11.5	0.95		0.7	1.6	98.4	0	125.0	7000	3.0	210	35.5	2490	2.5	180	59.0	4130	10	46	34	10	0	0

TABLE III
Sternal Marrow Pictures of Case 5 B. M.

	1951 8-20	9-12 10:00 A.M.	" 3:00 P.M.	9-14 10:00 A.M.	9-19	9-26	10-10	10-24	10-31
Erythropoiesis									
Megaloblasts	8.4	13.4	10.0	2.8	2.6	1.8	—	—	—
Macroblasts	18.6	16.2	6.6	24.1	8.0	4.0	1.8	3.0	2.0
Normoblasts	39.0	12.8	14.6	24.1	25.0	11.6	7.4	9.4	10.2
Mitosis	0.6	1.0	0.4	1.5	0.8	0.4	—	0.2	0.2
Granulopoiesis									
Myeloblasts	0.6	0.4	0.6	0.3	0.2	1.6	0.2	2.0	0.8
Promyeloblasts	1.8	10.0	3.6	3.2	0.8	0.6	2.2	1.2	3.6
Myelocytes	8.8	18.8	16.0	1.5	10.0	9.8	8.2	4.4	6.4
Metamyelocytes	18.0	19.2	16.4	9.1	20.0	18.2	33.0	15.8	20.2
Segmented form	4.0	2.8	9.8	3.7	4.4	13.6	11.6	7.6	12.0
Eosinophiles	5.2	2.2	5.2	6.5	2.8	4.2	3.2	6.8	3.8
Basophiles	—	—	0.2	—	—	—	—	—	—
Monocytes	—	—	0.4	1.5	0.4	0.4	0.8	1.4	0.6
Mitosis	—	0.8	0.2	—	0.4	0.4	0.2	0.2	0.2
Reticulum cells and lymphocytes									
Reticulum cells	0.8	—	0.2	1.9	4.6	1.2	—	4.0	4.8
Lymphocytes	1.8	0.2	9.6	6.3	—	27.0	26.8	36.0	29.0
Mitosis of reticulum cells	0.6	0.4	0.6	—	0.2	0.2	—	0.4	0.4
Erythroid myeloid ratio	1.7/1	1/1.2	1/1.7	1.7/1	1/1.1	1/2.8	1/6.5	1.3/1	1/3.8

TABLE IV
Peroxidase Pictures of Bone Marrow, Case 5. M.B.

Subject Day of observation 1951	Peroxidase activity									Remarks
	Granulocyte series			Erythroblast series			Erythrocyte in marrow			
	++ %	+	—	++ %	+	—	++ %	+	—	
Sept. 12 10:00 A.M.	12.5	19.3	68.2	6.1	12.1	81.8	11.0	88.0	1.0	Sternal puncture was performed immediately before the first injection of B ₁₂ . Five hours after the first intramuscular injection of 15 γ of B ₁₂ .
ditto 3:00 P.M.	10.9	21.1	68.0	32.3	32.3	35.3	28.0	71.8	0.2	
Sept. 14 10:00 A.M.	13.5	25.1	60.7	14.9	42.4	42.7	31.4	68.6	0	Strongly peroxidase-positive megakaryocytes were seen. Etracellular nuclei of erythroblasts were found either strongly peroxidase-positive or peroxidase-negative.
Sept. 19 10:00 A.M.	6.5	21.7	71.9	20.0	38.9	37.1	—	—	—	
Sept. 26	27.4	17.8	54.8	3.7	29.6	66.7	5.4	94.6	0	
Oct. 3	36.6	27.3	36.1	11.9	27.0	61.1	16.4	83.4	0.2	
Oct. 10	11.7	33.0	55.3	5.5	17.2	77.3	1.6	98.2	0.2	
Oct. 24	15.6	26.0	58.4	0	8.7	91.3	0.2	99.2	0.6

Fig. 5. (Case 5)
“Peroxidase Response” in the blood

Fig. 6 (Case 6)
“Peroxidase Response” in the bone marrow

was found in a complete parallelism with the reticulocyte reaction in Cases 3 and 5.

Basing on the above described finding, I desire to suggest the name of “Peroxidase Response” to this phenomenon—the critical increase of peroxidase activity of erythrocytes in anemic cases. It is well known

Fig. 7. "Peroxidase Response" of erythrocytes in the blood. (Case 5).
A) Before specific treatment. B) On the third day of commencement of vitamin B₁₂. Note a critical increase of strongly peroxidase-positive erythrocytes.

that the reticulocyte response is the very earliest sign of blood regeneration. But the "Peroxidase Response" can, it seems, occur at a much earlier stage than the reticulocyte response, as will be seen from the results of my own cases. As described previously, in my own case of megaloblastic anemia (Case 6), in which an apparently natural remission had occurred, "Peroxidase Response" of erythrocytes seems to have occurred a few days earlier than the reticulocytosis which occurred later. In my own case of pernicious anemia (Case 5), which had been treated quite effectively with vitamin B₁₂, the restoration of the normal developmental and proliferative activity of megaloblasts was ushered in by a remarkable "Peroxidase Response" of the cytoplasm or/and nuclei of the cells, which had presented itself within five hours after the commencement of the specific therapy. The "Peroxidase Response" of erythrocyte in the blood of this case seems to have occurred at the same time as that of megaloblasts in the bone marrow, although the former was less evident than the latter.

The peroxidase reaction of granulocytes, mainly myelocytes and metamyelocytes, was also intensified in this case (Case 5), which was confirmed by serial examinations. (Even some megakaryocytes—usually peroxidase-negative—were peroxidase-positive.) So a kind of "Peroxidase Response" occurred thus in the granulocytic system, I believe.

COMMENT

Recently, interests of some investigators have been focussed upon the alterations of activity or/ and concentrations of enzymes in erythrocytes of various types of anemia. Sabine³¹⁴⁾ (1940) found that the cholinesterase activity of the erythrocytes was greater than normal in various

Fig. 8. "Peroxidase Response" in the sternal bone marrow (Case 5). A) Before the commencement of specific treatment. Majority of nucleated elements were peroxidase-negative. The cells 2,4,6,10,11 and 13 are peroxidase-negative, 7,8 and 9 are peroxidase-positive erythroblasts (of megaloblast series); the cells 7 and 8 seemed to be in mitosis. The cells 1 and 3 are peroxidase-negative myelocyte and myeloblast respectively. The cell 12 is weakly peroxidase-positive erythrocyte and the figures 5 are probably aggregated platelets, weakly peroxidase-positive. B) A remarkable "Peroxidase Response" of megaloblasts at the fifth hour of the commencement of specific treatment. Note the striking increase of peroxidase granules in nuclei and cytoplasm of erythron (2,4,6,7,14,15 and 16). The cells 5 and 13 are weakly peroxidase-positive megaloblast and normoblast respectively. The cells 1 and 3 are peroxidase-negative myelocyte and weakly peroxidase-positive promyelocyte respectively. The cells 11 and 12 are strongly peroxidase-positive erythrocytes with weakly peroxidase-positive Howell-Jolly's bodies; the figures 8,9 and 10 are extracellular nuclei or nuclear remnants, some of which were strongly peroxidase-positive. . . these were found in the second to fourth week of specific treatment. C) and D) a kind of "Peroxidase Response" of granulocytes and of megakaryocyte, seen in the second and the third week of specific treatment. Note the conspicuous increase of peroxidase granules in neutrophile myelocytes (C1, D1 and 2), promyelocytes (C2), myeloblast (C3), band neutrophils (D3, 4) and segmented neutrophils (D5). The cell C4 is juvenile megakaryocyte, strongly peroxidase-positive.

anemias; in pernicious anemia in relapse the enzymic activity was normal or low, and during treatment it rose to high values like thoses of other anemias, then fell gradually as the blood picture approached normal. The author stated that the relative concentration of enzyme in the red cells was a more sensitive indicator of hyperactive hematopoiesis than the reticulocyte and that failure of elevation of enzymic concentration in severe anemia was associated specifically with suppression or malfunction of the hematopoietic system. Valee⁵⁾ (1949) described that patients with

pernicious anemia showed no decrease in absolute values for zinc and carbonic anhydrase activity in spite of marked lowering of hematocrit and hemoglobin levels and erythrocyte count. Accordingly, zinc concentration and carbonic anhydrase activity per unit of blood were elevated, often to a marked degree. These increases were parallel, varying inversely with the degree of anemia; when they regressed under treatment, both did so at the same rate.

Kerppola⁶⁾ (1951) reported that, if there was accelerated regeneration of blood, alkaline phosphatase was then present in increased amounts—even in the hemoglobin-containing red blood cells—in a definite and regular manner; and that, cases of pernicious anemia without spontaneous reticulocytosis showed no appreciable phosphatase content in the bone marrow or in the peripheral blood. If such a patient was given liver extract, an increase of alkaline phosphatase, in connection with accelerated regeneration of blood appeared. The reaction was mild in some cases; in others it was fairly intense. The phenomenon was observed shortly before the appearance of reticulocytosis and was most pronounced at the stage when reticulocytes increase rapidly. This is what he described.

Now, I described in the present paper the phenomenon of "Peroxidase Response" of erythron in connection with stimulated erythropoiesis. These data seem to support the thesis that morphological evidences of erythropoiesis seem to be produced by underlying enzymic actions. Consequently it appears reasonable that the determination of enzymic activity of erythron provides a sensitive indicator of bone marrow function.

The phenomenon of "Peroxidase Response" of erythron was a favorable sign from the prognostic viewpoint at least in the presented anemic cases. But, as I reported in the preceeding paper,¹⁾ high peroxidase levels of erythrocytes were found in various leukemic anemia too, and so it should be kept in mind that the prognostic significance of this phenomenon should be applied carefully and separately to each blood dyscrasia.

Remarks. The phenomenon of "Peroxidase Response" of erythron may forward a new problem to the study of anemia, though a further investigation and confirmation is necessary of course. In the present paper, "Peroxidase Response" of erythron is reported only preliminarily.

SUMMARY

In order to evaluate a hematological significance of the peroxidase reaction of erythrocytes or/and erythroblasts, six cases of various type of anemia i.e. one case of iron deficiency anemia of a weanling, one case of idiopathic thrombocytopenic purpura, one case of erythroblastosis

TABLE V

Date 1950	R.B.C. mill.	Hb g/dl	C.I.	Hb per 100 WBC	Retic. %	Peroxidase activity of erythrocytes				Sedim. Rate	Plt. thousands.	Bleed. time minutes	WBC	Bas.	
						++	+	-							
						%	%	%						%	absolt. count
Nov. 27	218	7.6	1.16	3	1.0					43(1hr.)	226	7.5	4850	0	0
28						91.3	8.7	0		71(2hrs.)					
Dec. 1	173	6.6	1.27	11	1.4						156		6700	0	0
4	198	6.3	1.06	38	14.4								7600	0	0
5	173	6.2	1.19		26.1										
6	258	7.7	0.99	30	38.1							6.0	7000	0	0
7	243	7.2	0.99		27.4										
9	348	8.0	0.77	10	22.8								5000	0.3	20
10	304	7.2	0.79		19.6										
12	380	9.0	0.79	4	10.3								6400	0.3	20
13	470	9.4	0.67		9.6										
14	466	10.1	0.72		5.9										
15	430	10.1	0.78	2	5.8						235	5.0	12000	0.5	60
16	415	10.1	0.80		7.1								20000	0.3	60
17	430	9.7	0.75	4	6.2							5.0	6100	0.8	50
18	410	10.4	0.85	2	9.4								7500	0.3	20
19	430	9.7	0.75	7	8.1								9600	1.0	100
20	435	10.4	0.80	14	8.2						224		20000	0	0
21	426	10.6	0.83												
22	390	9.7	0.85	5	9.0								7800	0.7	50
24	466	10.0	0.72												
25	498	10.7	0.72	5	7.2				4(1hr.)		348	3.5	7300	0	
27	536	10.7	0.67	5	2.8	23.0	77.0	0	8(2hrs.)		395		10500	1.3	140

Fig. 9 (Case 6)

Differential Count										Nuclear shift of neutrophils						
Eos.		Lym.		Plasm.		Mon.		Neut.								
%	absolt. count	%	absolt. count	%	absolt. count	%	absolt. count	%	absolt. count	M.	I	II	III	IV	V	VI
0.5	20	75.5	3660	0	0	1.0	50	23.0	1120	0	2	36	38	22	2	0
0.7	50	66.5	4460	0	0	3.3	220	29.5	1980	0	3	26	41	20	8	2
2.0	150	61.5	4670	0	0	12.0	910	24.5	1860	0	6	25	50	9	8	2
0.3	20	63.5	4450	0	0	5.5	390	30.7	2150	3	3	16	45	18	11	4
2.7	140	35.0	1750	0	0	16.0	800	46.0	2300	1	5	23	34	24	11	2
1.3	80	35.0	2240	0	0	14.0	900	49.0	3140	0	7	31	41	15	5	2
0.5	60	43.0	5160	0	0	12.0	1440	44.0	5280	1	18	42	33	5	0	1
0	0	11.0	2200	0.3	60	13.4	2680	75.0	15000	2	31	42	21	3	0	1
0	0	28.2	1720	0.4	20	11.8	720	58.8	3590	1	14	48	32	4	1	0
0.3	20	53.3	4000	0.7	50	7.7	580	37.7	2830	2	21	49	23	4	0	1
1.3	120	45.0	4320	0.7	70	9.0	860	43.0	4130	0	18	43	36	3	0	0
1.7	340	70.3	14060	0.6	120	5.7	1140	21.7	4340	0	8	40	38	14	0	0
6.3	490	26.7	2080	1.0	80	10.3	800	55.0	4290	0	3	25	45	24	2	2
7.5	550	51.0	3720	0.5	40	6.0	440	35.0	2560	0	5	38	36	17	4	0
4.7	490	48.3	5070	0.3	30	7.0	740	38.3	4020	1	5	27	48	17	2	0

TABLE VI
Sternal Marrow Pictures of Case 6 J. K.

	1950 11-29	12-6	12-15	12-20	12-25
Erythropoiesis					
Megaloblasts	2.8	1.8	0.1	0.7	0.6
Macroblasts	8.4	1.5	0.6	0.8	1.8
Normoblasts	57.2	67.4	25.4	30.5	22.4
Mitosis	2.0	1.9	0.2	0.8	—
Granulopoiesis					
Myeloblasts	1.6	0.8	2.4	0.2	0.2
Promyelocytes	2.8	1.6	4.6	3.9	2.0
Myelocytes	10.1	5.5	9.0	17.3	6.8
Metamyelocytes	17.6	5.8	28.2	19.0	27.6
Segmented forms	10.8	10.1	22.4	18.2	28.6
Eosinophils	0.5	0.4	15.0	1.8	0.8
Basophils	0	0.1	0.6	0.3	0
Monocytes	0	0.8	0.4	0.1	1.4
Mitosis	0.2	0.2	0.2	0.5	0.2
Reticulum cells and lymphocyte reticulum cells					
Reticulum cells	0.5	0.4	0	0.4	1.6
Lymphocytes	0.8	2.6	5.4	5.5	6.0
Erythroid-myeloid ratio	2.5/1	2.5/1	1/2.6	1/1.8	1/2.8

fetalis due to Rh incompatibility, one case of anemia due to intestinal hemorrhage, one case of pernicious anemia and one infantile case of megaloblastic anemia, were observed under the serial examination by combined use of the new technique of counting chamber method of peroxidase reaction devised by the author and of other routine hematological procedures. The results may be summarized as follow;

1. A certain regularity was noticed in the relationship between the peroxidase activity of erythrocytes or/and erythroblasts on one hand and the hemopoietic activity of erythron on the other. The peroxidase activity of erythron was greatly stimulated, in other words the critical increase of strongly peroxidase-positive erythrocytes in blood and erythroblasts in bone marrow developed at an early stage of blood regeneration.

2. To describe this remarkable phenomenon—the striking increase of strongly peroxidase-positive erythron at an early stage of blood regeneration—the author suggested the designation “Peroxidase Response” of erythron.

3. “Peroxidase Response” of erythrocytes or/and erythroblasts was a favorable sign, for the course of anemia of the presented cases.

4. It was surprising how rapidly the phenomenon had occurred in a case of pernicious anemia, i.e. the “Peroxidase Response” of megaloblasts in the bone marrow developed within five hours of the commencement of the specific therapy, and that of erythrocytes in the blood was observed shortly before the appearance of reticulocytosis.

5. In an infantile case of megaloblastic anemia, a marked peroxidase response of erythrocytes in blood seemed to occur a few days earlier than the following reticulocyte response.

6. As had been shown in one of my own cases, the determination of peroxidase level of erythrocytes or/and erythroblasts may be useful as a bone marrow function test and consequently as a test of the potency of therapeutic agents for anemic cases.

CONCLUSIONS

The phenomenon of “Peroxidase Response” of erythrocytes or/and erythroblasts, i.e. a critical increase of strongly peroxidase-positive erythrons, was described as a sign of blood regeneration on the basis of serial examination of 6 cases of various kind of anemia, including one case of pernicious anemia.

The “Peroxidase Response” was suggested for this striking increase of strongly peroxidase-positive erythrocytes in blood or/and erythroblasts in bone marrow in cases of anemia specifically treated. It could occur earlier than the reticulocyte response.

The counting chamber peroxidase method may thus provide a useful bone marrow function test, as well as a test of the potency of a therapeutic agent for anemic cases.

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