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Research Article

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Variations of Human Blood Cell Zinc in Disease *

ROBERT E. FREDRICKS, KOUICHI R. TANAKA, AND WILLIAM N. VALENTINE

(From the Department of Medicine, University of California Medical Center at Los Angeles, and the Wadsworth Hospital, Veterans Administration Center, Los Angeles, Calif.)

Variations in the amount of zinc in human blood cells have been correlated with several diseases and morphological features. In 1949, Vallee and Gibson observed that in pernicious anemia erythrocyte zinc is elevated to a degree even exceeding what might be related to macrocytosis (1). A correlation of carbonic anhydrase activity with erythrocyte zinc content was demonstrated (2, 3). Subsequent reports concerning erythrocyte zinc have shown that it is decreased in the fetus and newborn (3) and is increased in some patients with chronic leukemias and sickle-cell anemia (1, 2, 4–8). The first evidence concerning leukocyte zinc abnormalities was reported in 1949 by Vallee, Gibson, Fluharty, and Nelson (1, 4), who noted markedly decreased zinc in leukocytes from patients with chronic leukemias. They also described a rise in leukocyte zinc towards normal when subjects with chronic granulocytic leukemia responded to therapy. We have reported on the decreased amount of zinc in leukocytes of patients with hepatic cirrhosis (9), and high zinc levels in eosinophils have been described (10, 11).

This study was undertaken to evaluate the levels of erythrocyte and leukocyte zinc in a large group of patients with a variety of hematological and nonhematological diseases and to examine these cellular zinc levels in relationship to peripheral blood cell morphology, blood counts, leukocyte alkaline phosphatase activity, disease state, and treatment status. Included in the study are patients with acute and chronic leukemias, polycythemia rubra vera, myeloid metaplasia, megaloblastic and other anemias, eosinophilia owing to various causes, and pneumonia, and patients in the postpartum state.

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Methods

The methods of obtaining cells, preparing erythrocyte- and leukocyte-rich suspensions, and measuring the acid-extracted zinc in a dithizone and carbon tetrachloride solution have been previously described (9, 12). In most cases duplicate determinations were made on each sample, and the duplicate values were averaged, so that a single result, corrected for erythrocyte or leukocyte contamination, was recorded for each sample studied.

Leukocyte alkaline phosphatase was determined according to the method of Valentine and Beck (13). Standard methods were used to measure the hematological parameters, except that total blood cell counts were done with a Coulter counter and randomly checked by visual counts in hemocytometers.

The usual clinical and laboratory criteria were used in establishing diagnoses, and each case was reviewed by at least two of us, so that equivocal cases would be excluded. In this report the term "acute leukemia" includes all those cases that might be classified as acute or subacute leukemia. Included in the groups labeled as untreated are patients who have had no treatment for their disease for a period of at least 6 months, as well as those who have never been treated.

The range and mean values for zinc, in each instance recorded here, are the range and mean for all determinations within the group. If serial values for each patient are averaged and the mean value then determined, the result does not vary significantly from that calculated for all determinations.

Results

Values in patients with leukemias. Leukocyte and erythrocyte zinc determinations were performed on samples from 12 patients with chronic lymphocytic leukemia, 22 with chronic granulocytic leukemia, 3 with acute lymphocytic leukemia, 12 with acute monocytic leukemia, and 7 with acute granulocytic leukemia. The results are tabulated in Table I.

In all the groups of patients with leukemia the range of values and mean values for leukocyte zinc are less than normal, with the lowest values in chronic leukemia and acute lymphocytic leukemia and intermediate values in acute mono-

TABLE I

Blood cell zinc values in leukemia

Diagnosis	No. of determinations	No. of patients	Leukocytes			Erythrocytes		
			Range	Mean	SD	Range	Mean	SD
			μg Zn/10¹⁰			*μg Zn/10¹⁰*		
Normal	38	32	56.8–168	103.0	25.5	9.3–15.5	12.1	1.51
Chronic lymphocytic leukemia	15	12	0 – 78.7	52.2	20.0	7.7–15.9	13.0	2.10
Untreated	7	7	0 – 78.7	50.3	26.4	7.7–15.9	12.7	2.54
Treated	8	6	33.8– 71.3	53.8	11.8	10.3–15.5	13.3	1.97
Chronic granulocytic leukemia	36	22	17.8–130	58.7	22.2	7.0–18.5	13.3	2.65
Untreated	8	8	20.0– 64.3	48.4	15.7	10.4–17.3	14.0	2.13
Treated	28	19	17.8–130	62.1	22.8	7.0–18.5	13.1	2.77
Acute lymphocytic leukemia	4	3	34.3– 74.5	58.5	16.7	13.3–18.8	16.4	2.41
Acute monocytic leukemia	16	12	52.6–103	80.6	13.9	9.9–16.8	13.1	1.73
Acute granulocytic leukemia	7	7	43.9–122	94.4	26.6	7.4–13.2	11.8	1.84

cytic and acute granulocytic leukemia. If results are evaluated within each group of patients, no correlation of leukocyte zinc with total or differential leukocyte counts can be demonstrated (Tables II and III). Correlation coefficients indicate the lack of correlation between total leukocyte counts and leukocyte zinc; in chronic granulocytic leukemia r is minus 0.558, in chronic lymphocytic leukemia r is minus 0.254, in acute granulocytic leukemia r is plus 0.522, in acute monocytic leukemia r is plus 0.217, and in acute lymphocytic leukemia r is plus 0.101. The independence of leukocyte zinc values and the differential leukocyte counts is further emphasized

TABLE II

Blood cell zinc values in patients with chronic lymphocytic leukemia

Patient	Age Race Sex	Date	Leukocytes	Leukocytes*	Mature lymphocytes†	Immature lymphocytes‡	Granulocytes§	LAP‖	Erythrocytes	PCV¶	Treatment
			μg Zn/10¹⁰		%	%	%		*μg Zn/10¹⁰*		
A.B.	47, W, M	2/18	46.1	50,320	97.0	0.5	2.5	7.8	14.3	33	On prednisone
T.B.	59, N, M	12/18	42.9	191,000	91.0		9.0		7.7	39	None
J.D.	58, W, M	9/16	31.3	140,000	95.5		4.5		14.7	47	None
R.D.	82, W, F	11/9	64.0	156,000	80.5	4.0	15.5		14.3	29	X ray to spleen
		12/11	62.3	129,000	80.0	2.0	18.0	4.4	10.3	19	On chlorambucil
H.H.	63, W, M	1/26	49.5	38,200	30.5	49.0	16.0	6.7	15.5	23	On 6-mercaptopurine
H.K.	67, W, M	10/22	73.6	89,400	84.5		14.5		13.7	39	None
J.L.	64, W, M	3/22	0	450,000	99.5		0.5		12.3	29	None
F.P.	63, W, M	6/2	71.3	30,630	77.0	1.0	21.5		12.0	48	On prednisone
J.R.	61, W, M	5/8	43.3	112,000	97.0		3.0		13.6	42	On triethylenemelamine
		5/14	59.9	118,000	94.0		6.0		14.6	43	On triethylenemelamine
V.S.	60, W, M	5/27	51.5	175,000	91.5	2.0	4.5		10.8	34	None
		6/3	33.8	340,200	91.5	0.5	6.5		11.4	31	On prednisone and chlorambucil
C.T.	62, N, M	5/21	74.1	31,000	67.0		29.0		13.8	42	None
L.W.	65, W, M	2/18	78.7	35,900	89.5		10.0	15.6	15.9	29	Only transfusions for 10 months; previous X ray, triethylenemelamine, chlorambucil

* Leukocytes per cubic millimeter in peripheral blood.
† Percentage of mature lymphocytes in peripheral blood.
‡ Percentage of prolymphocytes and lymphoblasts in peripheral blood.
§ Percentage of all granulocytes in peripheral blood.
‖ Leukocyte alkaline phosphatase.
¶ Packed cell volume or hematocrit.

TABLE III

Blood cell zinc values in chronic granulocytic leukemia

Patient	Age Race Sex	Date	Leukocytes	Leukocytes*	Neutrophils			Eosinophils†	Basophils†	LAP§	Erythrocytes	PCV‖	Treatment
					Segmented†	Band†	Immature‡						
			μg Zn/10¹⁰		%	%	%	%	%		μg Zn/10¹⁰		
M.A.	57, W, F	6/18	50.0	38,300	52.0	8.0	18.0			1.3	13.8	32	On prednisone
P.A.	15, W, M	1/26	58.0	162,000	37.0	17.0	33.0	5.5	6.0	2.5	13.9	37	None
		2/23	108.	21,700	55.5	4.5	3.5	4.0	17.5	0.2	12.6	42	On busulfan
R.A.	54, W, M	3/1	33.5	491,000	22.0	13.0	47.5	12.0	3.0	0	12.5	27	None
		3/24	56.3	366,000	25.0	26.5	35.5	5.5	5.0	1.0	12.0	30	On busulfan
		5/4	29.4	69,000	46.0	19.0	20.5	4.5	3.0		12.7	40	On X ray to spleen
R.B.	40, W, M	2/23	61.9	26,100	50.0	14.5	13.0	2.5	9.0	3.3	10.6	48	On busulfan
A.D.	35, W, M	2/19	130.0	11,800	28.0	12.0	25.5	5.5	13.5	11.3	8.9	32	On busulfan
L.D.	40, W, F	1/15	20.0	210,000	42.5	21.0	31.5	4.0	1.5	0	11.7	40	None
J.E.	41, N, M	2/19	77.1	7,681	53.5	5.5	10.5		3.0	1.6	7.0	44	None 2 months; previous busulfan
E.G.	57, W, F	1/15	62.5	162,000	33.5	19.0	35.0	4.5	6.0	1.8	13.3	39	None
		2/24	17.8	85,890	43.0	25.0	10.5	2.0	12.0	2.9	14.1	39	On busulfan
		3/1	56.3	49,900	47.5	23.0	17.0	4.5	6.0	0	9.5	42	On busulfan
		4/12	79.9	6,850	66.0	4.0	3.0	7.0	1.0		12.8	44	On busulfan
K.G.	77, W, M	5/12	64.3	23,020	76.5	2.0	1.0	2.5	6.0		17.3	46	None
C.H.	62, W, M	3/15	25.2	9,849	63.0	9.0	4.5	0.5	1.0	24.1	18.5	37	On prednisone; busulfan 1 year ago
L.H.	43, W, M	1/14	58.2	12,260	80.0	5.0	11.0		1.0		13.8	40	X ray to spleen
		10/19	42.8	42,600	38.0	25.0	31.0	2.0	1.0	0	16.4	29	On busulfan; previous X ray to spleen
		12/12	45.3	106,000	41.5	29.5	32.0		1.0		11.6	28	None past month; previous busulfan and X ray to spleen
A.K.	45, W, M	1/14	77.2	89,800	37.5	15.0	35.5	1.5	3.5		15.9	45	X ray; previous busulfan, X ray, and colcemide

* Leukocytes per cubic millimeter of peripheral blood.
† Percentage in peripheral blood.
‡ Percentage promyelocytes and myeloblasts in peripheral blood.
§ Leukocyte alkaline phosphatase.
‖ Packed cell volume or hematocrit.

TABLE III—(*Continued*)

Patient	Age Race Sex	Date	Leukocytes	Leukocytes*	Neutrophils			Eosinophils†	Basophils†	LAP§	Erythrocytes	PCV‖	Treatment
					Segmented†	Band†	Immature‡						
			μg Zn/10¹⁰		%	%	%	%	%		μg Zn/10¹⁰		
		11/1	35.8	301,000	43.5	17.0	29.0	2.0	6.0	1.5	10.4	25	None 5 months; prior X ray, busulfan, and colcemide
		11/18	47.6	21,200	28.0	20.5	38.0	2.0	9.5	5.2	12.5	31	Splenic X ray; see above
M.L.	44, W, F	1/7	63.0	109,000	42.5	12.0	25.5	6.5	7.0	1.8	12.5	30	None 6 weeks; prior busulfan
		2/9	81.2	25,400	61.0	1.0	2.5	5.5	22.0	3.3	12.0	32	On busulfan
J.M.	46, W, M	6/5	58.7	61,890	36.5	15.5	32.5	0.5	2.0	16.1	12.0	30	On busulfan
L.M.	50, W, F	2/17	82.1	32,000	54.0	8.0	16.0	1.0	2.5	7.9	10.2	40	On busulfan
P.M.	72, W, M	1/11	54.3	59,000	5.0	2.0	91.5			4.4	13.8	33	On 6-mercaptopurine
D.N.	47, W, M	6/25	52.7	80,640	5.5	2.0	11.0	0.5	76.0	2.0	16.4	25	On busulfan
L.P.	41, N, M	5/8	66.6	196,400	32.0	15.0	44.0	5.0	1.0		18.5	31	On busulfan
W.P.	65, W, M	1/22	63.4	210,000	19.0	18.0	46.0	4.0	11.0	3.9	15.3	24	None
		2/18	74.2	16,700	26.0	18.0	30.5	3.0	9.5		17.1	27	On busulfan
		3/7	52.2	73,310	28.5	16.0	31.0	4.5	14.5	0	14.8	31	On busulfan
G.S.	24, W, M	6/9	37.6	24,900	54.0	8.5	15.5	3.0	2.0	28.3	15.5	43	None
R.S.	15, W, M	11/24	70.6	79,100	38.5	15.0	39.5	2.5	2.5	3.6	10.4	21	On colcemide; recent 6-mercaptopurine
W.T.	75, W, M	5/21	60.7	66,440	36.5	16.0	40.0	1.5	1.5	7.4	16.1	25	None 6 months; past busulfan and X ray
		9/29	58.6	164,000	22.5	2.5	64.0	5.5	0.5	3.1	12.2	24	On 6-mercaptopurine; past busulfan and X ray

by the similarity of zinc values for chronic leukemia and acute lymphocytic leukemia and by the fact that leukocyte zinc values for acute granulocytic leukemia are intermediate between normal values and those of chronic granulocytic leukemia.

In the leukemic patients the erythrocyte zinc values have a broader than normal range, and the mean values are higher than normal within every group except that of acute granulocytic leukemia. The degree of anemia, total and differential leukocyte counts, and treatment status do not correlate in any way with the erythrocyte zinc, and no definite relationship to the mean corpuscular volume of the erythrocytes could be established.

Values in patients with chronic lymphocytic leukemia. There is no significant difference between the leukocyte zinc levels of the treated and untreated groups with chronic lymphocytic leukemia. Although there is no definite correlation of leukocyte zinc with the total leukocyte counts in this group, it may be significant that no zinc was recovered from the leukocytes of the patient with the highest leukocyte count (450,000 per mm^3), whereas the greatest leukocyte zinc levels were found in the cases that had the lowest total leukocyte counts (Table II).

Values in patients with chronic granulocytic leukemia. The data for the chronic granulocytic leukemia group are remarkable in that leukocyte zinc shows a rise with response to treatment, as demonstrated by the difference in mean values for leukocyte zinc between the treated and untreated cases (Table I) and also by the individual responses of patients for whom serial determinations were done (Table III). Patient P.A. had the most remarkable rise of leukocyte zinc with a good response to busulfan. R.A. had an increase of leukocyte zinc with a response to busulfan, but while he was receiving splenic irradiation the zinc value was below the initial level. In E.G. leukocyte zinc first declined with busulfan therapy, but rose when good control was achieved with this drug. L.H. demonstrated his highest leukocyte zinc value while in his best remission with X-ray therapy to the spleen. A.K.'s lowest leukocyte zinc value was noted when he was not being treated and was "out of control," and the value rose when he responded to splenic X-ray treatment. M.L. responded well to busulfan therapy and had a concurrent elevation of his leukocyte zinc. W.P.'s highest leukocyte zinc value occurred while he was having his best response to busulfan. The information for W.T. is inconclusive.

Although the generalization about the lack of correlation of leukocyte zinc with total leukocyte counts applies to the group with chronic granulocytic leukemia, the highest leukocyte zinc values were found in the cases with the lowest counts, and the converse is also true. In the twelve determinations done on samples of peripheral blood with total leukocyte counts of 20,000 per mm^3 or less, leukocyte zinc values ranging from 37.6 to 130 μg per 10^{10} leukocytes were found, whereas the range was 20 to 66.6 μg per 10^{10} leukocytes in the eleven instances when the total leukocyte count was 100,000 per mm^3 or greater (Table III). This may not be an exception to our earlier generalization, but rather another reflection of the effect of treatment on leukocyte zinc in chronic granulocytic leukemia.

Values in acute leukemia. A summary of findings in each type of acute leukemia is given in Table I. The acute lymphocytic and acute granulocytic leukemia groups contain only a single value for leukocyte zinc in an untreated patient; therefore, no judgment of the effects of treatment on

TABLE IV

Blood cell zinc values in polycythemia rubra vera and myeloid metaplasia

Diagnosis	No. of determinations	No. of patients	Leukocytes			Erythrocytes		
			Range	Mean	SD	Range	Mean	SD
			$\mu g\ Zn/10^{10}$			$\mu g\ Zn/10^{10}$		
Normal	38	32	56.8–168	103.0	25.5	9.3–15.5	12.1	1.51
Polycythemia	17	13	59.0–232	108.1	44.4	5.5–14.6	11.3	2.59
Untreated	6	6	59.0–232	116.2	59.2	7.3–13.9	11.5	2.09
Treated	11	10	59.5–143	103.8	32.9	5.5–14.6	11.2	2.92
Myeloid metaplasia	8	6	42.3–108	68.8	22.1	11.9–19.8	14.9	2.79

TABLE V

Blood cell zinc values in polycythemia rubra vera and myeloid metaplasia

Patient	Age Race Sex	Date	Leukocytes	Leukocytes*	Neutrophils: Segmented†	Neutrophils: Bands†	Neutrophils: Immature‡	Eosinophils†	Basophils†	LAP§	Erythrocytes	PCV‖	MCV¶	Treatment
			μg Zn/10^{10}		%	%	%	%	%		μg Zn/10^{10}			
Polycythemia rubra vera														
R.A.	48, W, M	2/24	86.9	20,400	76	14		2.5	1.0		11.8	50	92	P_{32}
J.B.	63, W, M	6/30	85.6	9,430	44	2		2.0	2.0		11.9	54	92	Phlebotomies
S.B.	59, W, M	2/5	232.0	11,600	77	3		1.0			7.3	82	74	None
		2/9	167.0	11,900	70.5	4.5		2.0			5.5	76	76	Phlebotomies
W.C.	43, W, M	2/2	59.0	14,800	83.0				0.5	61	12.3	54	95	None in 10 months; previous phlebotomies and P_{32}
E.Gar.	58, W, F	1/10	71.0	21,250	78.0	2.0		3.0	2.0		8.9	61	79	Phlebotomies
		10/18	90.4	33,800	80.0	1.5		1.0	2.0		13.9	55		None 8 months; prior phlebotomies
		11/22	65.9	32,100	76.5	5.5		2.0	1.0	214.38	14.1	48		Phlebotomies
E.Gad.	50, W, M	2/16	107.0	32,200	83.0	6.5		4.5	1.5	303.74	10.7	68	81	None 14 months; prior phlebotomy, P_{32}, X ray
H.K.	62, W, M	6/12	63.9	13,260	78.0	7.0		4.0			12.5	45	82	None 5 years; prior phlebotomies and P_{32}
F.M.	45, W, M	3/24	97.8	15,000	74.0			1.5		43.24	12.6	53	80	Phlebotomies
J.Q.	49, W, M	12/1	106.0	9,225	60.0	0.5		1.5		22.80	13.1	48	94	Phlebotomies
L.R.	63, W, M	2/17	59.5	15,300	75.0	5.0		1.0	1.0	261.23	6.8	61	81	P_{32}
J.R.	74, W, M	2/11	129.0	22,300	75.5	2.0	0.5	6.5	2.5		11.0	62	72	Phlebotomies
E.S.	65, W, F	1/8	129.0	19,400	81.5	5.0	0.5	4.5	0.5	164.0	13.2	54	73	Phlebotomies
M.S.	75, W, M	5/27	143.0	16,230	54.0	1.0		3.0		40.0	14.6	54	94	Phlebotomies
		12/11	145.0	10,700	64.0	1.5		5.5	1.0	86.0	12.2	55		None 6 months, previous phlebotomies
Myeloid metaplasia														
W.C.	71, W, M	2/5	91.5	48,200	22.5	26.5	33.5	1.0	2.5	0	12.0	29	93	On prednisone
E.H.	64, W, F	6/19	57.9	28,460	65.5	14.5	9.5	1.0	2.5	263.0	12.5	55		None 9 weeks; past busulfan
		12/18	48.7	17,200	76.0	9.0	4.5	1.0	0.5	274.0	13.0	40	87	On busulfan
M.H.	71, W, F	7/10	42.3	50,080	44.0	13.0	35.0	3.0	3.0	112.0	12.0	40	81	On busulfan
R.K.	46, W, M	4/6	68.0	16,300	68.0		16.5	2.5	3.5	84.0	19.8	26	124	Transfusions
H.S.	69, W, M	2/28	49.4	27,500	77.0	2.0		2.0	1.0	30.0	16.3	32	89	None 2 months; past transfusions
		9/24	108.0	36,500	60.0	10.0		5.0	1.0	24.0	17.9	33	91	Transfusions
O.T.	70, W, M	1/8	84.3	42,500	43.0	16.5	35.5			47.0	16.0	25	93	On prednisone and busulfan

* Leukocytes per cubic millimeter of peripheral blood.
† Percentage in peripheral blood.
‡ Percentage promyelocytes and myeloblasts in peripheral blood.
§ Leukocyte alkaline phosphatase.
‖ Packed cell volume or hematocrit.
¶ Mean corpuscular volume of erythrocytes.

leukocyte zinc can be made in these cases. Studies on cases of acute monocytic leukemia included determinations of blood cell zinc of four patients who had never received treatment for their disease; however, the data do not show any relationship of blood cell zinc to treatment.

Values in polycythemia rubra vera and myeloid metaplasia. Thirteen patients with polycythemia rubra vera and six who had myeloid metaplasia are included, and the results are shown in Table IV. In all but one of the seventeen determinations done on samples from polycythemic patients, the leukocyte zinc values are normal. The remarkably elevated value of 232 μg of zinc per 10^{10} leukocytes was found in a blood sample from an untreated patient when he had a packed cell volume of 82% (Table V). Four days later, after many phlebotomies had reduced this to 76%, the leukocyte zinc had fallen to 167 μg per 10^{10} leukocytes. All the other values for the polycythemia group fall into the normal range, have a normal mean value, and show no relationship to total (r, -0.297) or differential leukocyte counts, packed cell volume, or treatment status.

In the cases of myeloid metaplasia leukocyte zinc is significantly decreased, and, again, without relationship to total leukocyte counts (r, 0.333), differential leukocyte counts, or hemoglobin levels. The available data do not permit evaluation of the effects of treatment on zinc levels.

The erythrocyte zinc levels in both polycythemia rubra vera and myeloid metaplasia seem to show a direct relationship to mean corpuscular volumes (MCV) of the erythrocytes. The highest MCV of 124.4 μ^3 is associated with the highest erythrocyte zinc value of 19.8 μg per 10^{10} erythrocytes as seen in Table V.

Zinc values related to leukocyte alkaline phosphatase. In many of the cases of leukemia, polycythemia, and myeloid metaplasia, leukocyte alkaline phosphatase activity was measured on portions of the blood samples obtained for zinc determinations (Tables II, III, and V). The results clearly show that levels of leukocyte alkaline phosphatase and values for zinc in leukocytes are completely independent of each other.

Values in anemia, eosinophilia, pneumonia, and postpartum cases. Three patients with megaloblastic anemia (two due to folic acid deficiency and one due to pernicious anemia) were studied. Pretreatment blood samples were analyzed in each case, and in each there was a markedly elevated erythrocyte zinc value. In the two cases of folic acid-deficiency anemia in which posttreatment blood samples could be obtained, the erythrocyte zinc decreased as MCV declined and the packed cell volume and hemoglobin increased (Table VI).

Sufficient leukocytes for zinc analysis could be obtained only from patient H.W. after his third week of folic acid therapy. The first leukocyte zinc value was significantly low, but the leukocyte zinc rose into the normal range after an additional week of treatment, when the erythrocyte zinc value had fallen to normal.

Patients with paroxysmal nocturnal hemoglobinuria had normal erythrocyte zinc in the four cases studied. Sufficient leukocytes for study could not be obtained from these patients because of the characteristic low leukocyte counts.

TABLE VI

Blood cell zinc values in megaloblastic anemias

Diagnosis	Patient	Age Race Sex	Date	Treatment	Leukocytes	Erythrocytes	PCV*	MCV†	Leukocytes‡
					$\mu g\ Zn/10^{10}$	$\mu g\ Zn/10^{10}$			
Folic acid deficiency	M.O.	38, W, F	4/22	None		19.7	27	120	6,200
Folic acid deficiency	M.O.		5/9	On folic acid		15.3	33	124	5,300
Pernicious anemia	F.R.	69, W. F	7/10	None		27.8	22		7,460
Folic acid deficiency	H.W.	41, W, M	7/10	None		26.8	14	175	9,310
Folic acid deficiency	H.W.		7/20	On folic acid		21.0	25	135	6,190
Folic acid deficiency	H.W.		8/3	On folic acid	35.4	16.7	33	111	5,600
Folic acid deficiency	H.W.		8/10	On folic acid	75.1	12.7	34	110	6,350

* Packed cell volume or hematocrit.
† Mean corpuscular volume of erythrocytes.
‡ Leukocytes per cubic millimeter of peripheral blood.

Eleven patients with other types of anemia (two thalassemia minor, three sickle-cell anemia, one SC disease, one fava bean sensitivity anemia in remission, two idiopathic acquired hemolytic anemias with remote splenectomies, one congenital hemolytic anemia with remote splenectomy, and one due to hereditary spherocytosis) had evaluations of leukocyte and erythrocyte zinc, but no definite abnormality was suggested by the results.

Seven patients with eosinophilia (ranging from 12.5 to 73.5% and associated with parasitic infestations, collagen diseases, drug reactions, and Loeffler's syndrome) were studied. Only one zinc value was outside the normal range, a low value of 47.1 μg of zinc per 10^{10} leukocytes in the patient with 73.5% eosinophils. The leukocyte zinc did not correlate with the differential leukocyte count or other measured values. A patient with 58.5% eosinophils had 101 μg of zinc per 10^{10} leukocytes, and another with 12.5% eosinophils had 59.9 μg of zinc per 10^{10} leukocytes.

Six patients with leukocytosis owing to pneumonia had normal blood cell zinc values, except for the one patient who also had cirrhosis. In the latter, leukocyte zinc was distinctly low, 29.2 μg of zinc per 10^{10} leukocytes.

Leukocyte and erythrocyte zinc values on samples from one woman during labor and from six during the immediate postpartum period were distributed in the normal range.

Discussion

Our studies (6) and those of Wolff (11) and of Dennes, Tupper, and Wormall (7, 8) have shown again that there is a decrease in the zinc content of leukocytes from persons with chronic lymphocytic and chronic granulocytic leukemias, as originally observed by Gibson and associates (4). In addition, this report points up that *a*) leukocytes of patients with acute lymphocytic leukemia and myeloid metaplasia also have a low level of zinc, whereas the leukocytes in acute monocytic and acute granulocytic leukemia contain about 80 to 95% of the normal amount of zinc, *b*) the leukocytes in polycythemia vera and leukocytosis contain zinc in normal amounts, and *c*) the leukocytes in megaloblastic anemia may be zinc deficient.

Attempts have been made to correlate variations in leukocyte zinc levels with the differential leukocyte counts, the degree of immaturity of the leukocytes, the total leukocyte counts, and various modes of therapy used in the diseases studied.

Wolff (11) has reported that eosinophils contain large amounts of zinc, and the histochemical studies of Mager, McNary, and Lionetti (10) confirm this characteristic; however, the values found in this study do not show any correlation of zinc content with the eosinophil counts in a group of seven patients with eosinophilia ranging from 12.5 to 73.5%. Extensive tables in this paper demonstrate further the great variations in differential cell counts present in cases with similar leukocyte zinc values. For example, there is a striking similarity between the zinc levels in chronic lymphocytic leukemia, where leukocyte samples contain almost pure lymphocytes, and in chronic granulocytic leukemia, where leukocyte suspensions contain variable percentages of neutrophilic forms, eosinophils, and basophils (most strikingly, 76% basophils as shown for D.N. in Table III).

Wolff (11), Candura and Candura (14), and de Nicola and Candura (15) have offered evidence that the leukocyte zinc level varies with cellular maturity, being lowest in the most immature cells. Wolff based his conclusion on the evaluation of cases of chronic leukemia, and the Italian workers included just one example of acute leukemia among the seven cases of leukemia that they used in graphing this relationship. A relationship between leukocyte zinc and cellular maturity is not apparent from the data detailed in Tables II, III, and V, and the most obvious argument opposing this concept is that the leukocyte zinc levels in acute granulocytic and acute monocytic leukemias exceed those found in cases with chronic leukemia, as shown in Table I.

An inverse relationship between the total peripheral leukocyte count and leukocyte zinc content in chronic lymphocytic leukemia has been reported by Dennes and associates (8). The data of this study do not reveal a similar clear-cut association, although leukocyte zinc values in chronic lymphocytic leukemia and also in chronic granulocytic leukemia are lowest in the cases that had the highest leukocyte counts, and greatest in the cases with the lowest total leukocyte counts. There is

no correlative association between total leukocyte counts of peripheral blood and leukocyte zinc values in the various other groups presented here, or in the previously reported study of patients with cirrhosis (9); the maximal correlation coefficients are plus 0.522 and minus 0.558.

As seen in Tables I and III, this study confirms the reports of Gibson and associates (4) and Wolff (11), who noted that leukocyte zinc concentrations rise in cases of chronic granulocytic leukemia which respond to therapy. A similar effect of therapy on leukocyte zinc could not be demonstrated for the patients with chronic lymphocytic leukemia, and insufficient information is available for such an evaluation of the effects of therapy in the other patients with leukemia and myeloid metaplasia. Although only one case has been studied, it seems clear that there was a rise of leukocyte zinc from a low to a normal level, as the patient with megaloblastic anemia owing to folic acid deficiency responded to treatment.

In 1952, Hoch and Vallee (16) described the extraction of a zinc-containing protein from leukocytes, which they estimated accounts for 80% of leukocyte zinc. The biological role and biochemical significance of this protein, and, indeed, of leukocyte zinc in general, have not been determined. It is assumed that this zinc metalloprotein of leukocytes is an enzyme or group of enzymes; however, Vallee (17) states that there is no correlation of zinc levels and alcohol dehydrogenase, carboxypeptidase, lactic dehydrogenase, or rhodanese activity in human leukocytes. Leukocyte alkaline phosphatase, which is, most likely, another zinc metalloenzyme (18), varies in activity without relationship to leukocyte zinc levels (9), as can be seen from the data presented in this report. Other leukocyte enzymes that have been studied in this laboratory, including glucose-6-phosphate dehydrogenase, fumarase, aconitase, arginase, and lactic dehydrogenase, have shown patterns of activity that would not correlate with the known variations of leukocyte zinc.

Previously published reports have described abnormalities of erythrocyte zinc levels, specifically elevations in pernicious anemia with return to normal as remissions are induced and high values in patients with chronic leukemias (1, 2, 4–8). The results reported here substantiate these findings and also those of Dennes and associates (8), who observed that the range of erythrocyte zinc values is much greater in chronic leukemic subjects than in normal subjects. In addition, we have found elevated erythrocyte zinc levels in patients with acute lymphocytic leukemia, acute monocytic leukemia, and myeloid metaplasia. We have previously commented on the broad range of erythrocyte zinc levels in patients with cirrhosis (9) and on the possible correlation of these values with the MCV of the erythrocytes; a similar correlation is suggested by the data for polycythemia vera and myeloid metaplasia tabulated in Table V. No other relationship of erythrocyte zinc to hematological determinations, disease state, or treatment status has been detected.

The direct relationship of erythrocyte zinc levels to carbonic anhydrase activity of the erythrocytes was first described by Vallee, Lewis, Altschule, and Gibson in 1949 (2). This close association has never been discounted; however, the study of Dennes, Tupper, and Wormall (19), in which they measured radioactive $zinc^{65}$ uptake by blood cells, has demonstrated that there is a portion of erythrocyte zinc which exchanges freely with the body pool of zinc and is apparently not complexed in any intimate chemical union with proteins of the cells, whereas the zinc of carbonic anhydrase is a closely held constituent of that enzyme and cannot be dialyzed out of the cell even with potent zinc chelating agents (8).

It is not likely that easily exchanged or "labile" zinc could contribute to the increased concentration of erythrocyte zinc reported in certain diseases. In the methods used in determining our data and that of other published reports concerning increased erythrocyte zinc, the "labile" portion of zinc in erythrocytes is probably removed during the cell washings (8) that precede the analysis of the zinc content. The measured erythrocyte zinc probably represents the "stable" zinc, the protein-bound zinc, which is incorporated in the carbonic anhydrase molecule. Previously published reports have shown that serum zinc levels are reduced in the clinical situations in which we find increased erythrocyte zinc values (20). This fact would lead one to postulate that the "labile" zinc in these erythrocytes would decrease, because of the tendency for the "labile" zinc of the cells to be equilibrated with the

Diagnosis	WBC Zn*	RBC Zn	Serum Zn
Folic acid deficiency anemia	30%	↑	↓
Chronic lymphocytic leukemia	50%	↑	↓
Chronic granulocytic leukemia	60%	↑	↓
Acute lymphocytic leukemia	60%	↑	
Myeloid metaplasia	70%	↑	
Cirrhosis	70%	N	↓
Acute monocytic leukemia	80%	↑	
Acute granulocytic leukemia	95%	N	
Polycythemia vera	100% to ↑	N to ↓	N to ↑
Anemias (non-megaloblastic)	100%	N	
Eosinophilias	100%	N	
Leukocytoses	100%	N	↓
Women at parturition	100%	N	↓

*WBC Zinc expressed as approximate percentage of normal mean value

N = normal value

↑ = value greater than normal mean value

↓ = value less than normal mean value

FIG. 1. SUMMARY OF THE RELATIONSHIPS OF LEUKOCYTE, ERYTHROCYTE, AND SERUM ZINC LEVELS IN VARIOUS DISEASES.

body zinc pool, as most immediately reflected in the erythrocyte's plasma environment.

Many of the findings discussed in this paper are graphically represented in Figure 1, which illustrates that there is a reciprocal relationship between leukocyte and erythrocyte zinc levels. Erythrocyte zinc tends to be increased in those clinical states in which leukocyte zinc is decreased, with the notable exception of the cases of cirrhosis in which the average erythrocyte zinc value is normal even though leukocyte zinc is decreased (9). Greatly elevated leukocyte zinc was found in only one instance, in a patient with polycythemia vera (S.B. in Table V), and his erythrocyte zinc was reciprocally decreased. It has also been reported that serum zinc may be increased in some cases of polycythemia vera (20). This single case then gives complementary strength to the many examples of a reciprocal relationship of leukocyte and erythrocyte zinc noted among the groups with decreased leukocyte zinc.

The abnormal leukocyte zinc levels may reflect long-standing disturbances in serum zinc. In cirrhosis serum zinc is depleted apparently because of the associated zincuria (21). Increased binding of zinc by erythrocytes may be responsible for reducing serum zinc in cases of megaloblastic anemia, chronic leukemia, acute lymphocytic leukemia, and myeloid metaplasia. Decreased serum zinc might be expected to be reflected first in the leukocytes, because they have a rapid turnover and also because they ordinarily take up and bind 30 to 75 times more zinc than erythrocytes. Cases of polycythemia rubra vera may provide us with a model for defining these interrelationships, since an occasional florid case has a reduction of erythrocyte zinc and an increase of serum zinc that may be responsible for the elevation of leukocyte zinc.

Summary

1) Leukocyte zinc values for 46 patients with various acute and chronic leukemias were found to be less than normal, and the lowest values

were noted in cases of chronic leukemia and acute lymphocytic leukemia.

2) The rise of leukocyte zinc content with response to therapy in chronic granulocytic leukemia is confirmed.

3) Leukocyte zinc was normal in 16 and elevated in one determination on samples from patients with polycythemia rubra vera, but significantly decreased in six patients with myeloid metaplasia.

4) In one patient with megaloblastic anemia, leukocyte zinc rose from a low level into the normal range with response to therapy and subsidence of the anemia.

5) Leukocyte zinc was normal in six patients with leukocytosis due to pneumonia.

6) Leukocyte and erythrocyte zinc levels were normal in seven women during labor or the immediate postpartum period.

7) Leukocyte zinc levels could not be correlated with eosinophilia, basophilia, or other variations in differential leukocyte counts or with total leukocyte counts or with the degree of leukocyte immaturity.

8) The level of leukocyte alkaline phosphatase activity shows no relationship to leukocyte zinc levels.

9) Erythrocyte zinc content is often increased in patients with chronic leukemias, acute lymphocytic leukemia, acute monocytic leukemia, and myeloid metaplasia.

10) As reported by others, we found that erythrocyte zinc is increased in megaloblastic anemia and declines with response to therapy.

11) In some cases the erythrocyte zinc content seems to correlate with the mean corpuscular volume of the erythrocytes, but in most cases shows no correlation with the degree of anemia or other factors.

12) There does seem to be a reciprocal relationship between leukocyte and erythrocyte zinc levels in various disease states, and the possible implications of this observation are discussed.

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