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Classification and Evolution of Patterns of Erythropoiesis in Polycythemia Vera as Studied by Iron Kinetics

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Diverse patterns of erythropoiesis have been described in polycythemia vera. Increased erythropoiesis is present, but patients may have normal (1, 2) or shortened red-cell life span (3, 4). Splenic sequestration of red cells and extramedullary erythropoiesis may or may not be present (3, 4, 5). A relative or absolute decrease in red-cell volume may occur, and some patients may die with a refractory anemia (3, 6). The present work is directed toward classification of the diverse patterns, and definition of the evolutionary history of erythropoiesis in this disease. Possible bases for the pattern of evolution of erythropoiesis documented in this work are discussed. Sixty-four patients with polycythemia vera were studied by means of iron kinetics. Seven of these patients had repeat iron-kinetic studies over a 12-year period. The clinical course and pathologic findings at postmortem examination on the patients were correlated with iron-kinetics findings.

MATERIALS AND METHODS

Except where indicated, patients reported in the series were diagnosed as having polycythemia vera on the basis of: a) an increased circulating red-cell volume exceeding 34 ml/kg body wt; and, b) exclusion of disorders associated with secondary erythrocytosis. Tumors associated with secondary erythrocytosis were excluded on the basis of complete medical evaluation during clinic visits and subsequent long-term follow-up of the patient's course and, when applicable, postmortem findings. Arterial blood-oxygen saturation was performed in patients where the possibility of chronic pulmonary disease or right to left cardiac and vascular shunts was present. All patients were evaluated by members of the medical group, and in most cases therapy and long-term follow-up were performed at the Donner Laboratory clinic. Complete records were kept of all pertinent medical information concerning procedures performed elsewhere throughout the entire course of the patient's disease.

Iron-kinetic studies were performed according to the techniques of Pollycove and Mortimer (7). Ten to forty microcuries of radioactive ^{59}Fe, ferrous citrate, specific activity 5-25 μCi per microgram, were used. This amount was added to sufficient fresh autologous plasma and the mixture incubated at 37°C for 20-30 min to insure binding of all iron to the beta-1 globulin transferrin. Five to twenty milliliters of the labeled fresh plasma were injected intravenously with a calibrated syringe. During the 14 days following injection, serial blood samples were analyzed for plasma and erythrocyte radioactivity and surface measurements of radioactivity over the liver, spleen and sacral bone marrow were made, as

1160692

described previously (7, 8). Plasma and erythrocyte radioactivities were measured by counting 2- or 3-ml samples in a well scintillation counter with 2-inch or 3-inch sodium iodide crystals and 6-inch or 4-1/2-inch lead shielding. The counter efficiency was approximately 16% for ^{59}Fe, the background approximately 20 counts per minute. All samples were counted for sufficient time periods to accumulate over 2,000 counts above background to insure satisfactory counting statistics. Blood samples were centrifuged at 2,000 g for 15 min twice to obtain plasma free of erythrocytes. It was necessary to take care in drawing and manipulating the blood to avoid hemolysis which, after significant labeling of erythrocytes occurs, would produce erroneously high concentration of radioiron in plasma. Concentration of iron in plasma was determined by the modification of Peters and co-workers of the Ramsey method (9). Hematocrits were determined on all samples (Wintrobe). Plasma and red-cell volumes were determined independently by the ^{59}Fe and ^{32}P dilution methods. Surface counting rates were obtained from mobile scintillation counters over liver, spleen and sacrum. External monitoring values are expressed as a fraction of the zero time extrapolate value.

Plasma-radioiron curves obtained over a 14-day period were analyzed for iron incorporation into hemoglobin by first correcting the curves for hemolytic feedback of iron into the plasma and then analyzing the resulting curves in the fashion previously described by Pollycove and Mortimer (7). Where there is neither hemolytic nor marked storage feedback of iron into the plasma, the radioiron-plasma-disappearance curve falls exponentially throughout the first 10- to 14-day period of observation. Therefore, in the absence of marked feedback from stores, it was assumed that whenever the plasma-radioiron curve reached an early asymptote during the first two-week period that this asymptotic value was primarily the result of radioiron feedback from hemolysis. By: a) assuming that extramedullary hemolytic feedback of radioiron into the plasma was proportional to the amount of radioiron incorporated in circulating erythrocytes; and, by b) assuming that the asymptote reached by the plasma-radioiron curve during the first two weeks was due primarily to hemolytic feedback of iron into the plasma, a curve could be drawn which approximates the extramedullary hemolytic feedback function of radioiron to plasma (see Fig. 2 and Fig. 4). When intramedullary hemolysis is present, causing the plasma-radioiron-clearance curve to reach an asymptote much earlier than the time of maximum incorporation of radioiron into erythrocytes, hemolytic feedback of radioiron into the plasma is estimated by a function having the shape of normal red-cell ^{59}Fe-incorporation curve but more rapid and displaced in time so as to coincide with the point at which the plasma radioiron reaches its asymptote (see Fig. 3). On subtraction of this hemolytic feedback curve from the measured plasma-radioiron concentration, a curve with two or three exponential components was obtained, which was analyzed in the fashion previously described by Pollycove and Mortimer.

Measurement of platelet survival was performed using ^{32}DFP in a manner described previously (10). White-blood-cell counts were performed in a routine fashion. Differential counts were done on Jenner-Giemsa stained air-dried blood films. Platelet counts were determined by the phase contrast technique. All cell counting was done in duplicate.

1160693

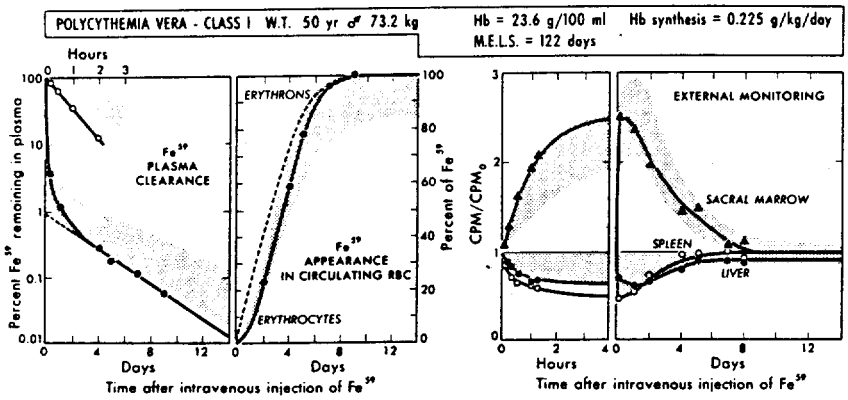

Figure 1. In polycythemia Class I ^{59}Fe kinetics, plasma-iron turnover is increased and normal patterns of radioiron incorporation into circulating red cells and external monitoring over sacral bone marrow, liver and spleen are seen. Analysis of plasma-radioiron data (7) yields increased values for rate of hemoglobin synthesis, but mean erythron life span is within normal limits (i.e., increased red-cell volume is proportional to increased erythropoiesis).

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RESULTS

^{59}FE-KINETICS PATTERNS: ERYTHROPOIETIC CLASSES I, IIa, IIb, III The iron-kinetics results obtained from studies of 64 patients with polycythemia vera (Table 1) can be classified into four distinct patterns. The first pattern is characterized by a normal RBC life span and is exemplified by the data and results shown in Fig. 1. External monitoring data are similar to that seen in normal subjects (7), with a rapid accumulation of iron in the bone marrow and subsequent complete release. There is no evidence of splenic sequestration of red cells nor any evidence of extramedullary hemopoiesis. The pattern of appearance of ^{59}Fe in circulating RBC is within normal limits. Plasma-iron turnover is increased above normal values (class average of nine patients not given previous radiation therapy from Table 2 is 0.040 mg/kg/hr ± σ = 0.016, normal being 0.019 ± σ = 0.004). Analysis of plasma-radioiron data obtained during a two-week period according to the kinetics model of Pollycove and Mortimer (7) shows increased hemoglobin synthesis (same class average on Table 2 is 0.172 g/kg/d ± σ = 0.046, normal being 0.089 ± σ = 0.014) and a normal mean erythron life span, (same class average from Table 2 = 118 days ± σ = ± 13.6 normal being 117 days ± σ = 7.5). This pattern is designated polycythemia Class I.

Class II is characterized by shortened red-cell survival in the absence of extramedullary erythropoiesis. This class is subdivided into two groups; Class IIa, in which red-cell hemolysis occurs after release from the bone marrow (usually with splenic sequestration and destruction); and Class IIb, in which the majority of red-cell hemolysis occurs in the narrow

1160694

Table I. Iron kinetics in polycythemia vera.

Patient	Sex	Age at time of Dx	Treatment before study: Rx before Fe^{59} kinetics	Treatment before study: Time before Fe^{59} study of last Rx (yr)	Maximum recorded RBC vol. (ml/kg) [24-33]	Time after Dx max. RBC vol. measured (yr)	Hematologic values at time of Fe^{59} kinetics (normal values): Time from Dx to study (yr)	WBC (No./mm^3) [5 000-18 000]	Platelets (No./mm^3) [250 000-500 000]	Hemoglobin (g/100 ml) [14.0-16.0]	RBC volume (ml/kg) [24-33]	Plasma volume (ml/kg) [32-45]	Serum iron (µg/100 ml) [70-170]	Plasma iron turnover (mg/kg/hr) [0.019±.006]	Calculated Hgb synthesis (g/kg/day) [0.069±.014]	Mean erythron life (days) [117±7.5]	Erythropoietic site: Marrow	Spleen	Liver	Time from Dx to death (yr)
Polycythemia vera Class I																				
[redacted]	M	[redacted]	None	--	59.4	1.2	1.2	5 900	290 000	21.0	56.8	32.2	85	0.020	0.124	139	+	0	0	--
[redacted]	F	[redacted]	None	--	48.4	0.0	4.3	60 600	360 000	18.2	48.4	33.7	89	0.044	0.116	112	+	0	0	--
[redacted]	M	[redacted]	None	--	90.3	0.9	0.5	8 400	224 000	23.6	85.8	24.2	230	0.061	0.225	122	+	0	0	14.9[a]
[redacted]	M_1	[redacted]	None	--	68.5	0.0	0.0	17 600	290 000	22.3	60.3	32.5	130	0.029	0.188	134	+	0	0	11.7
[redacted]	M	[redacted]	None	--	58.3	1.1	0.4	25 500	340 000	19.6	52.6	35.2	119	0.037	0.128	127	+	0	0	--[b]
[redacted]	M	[redacted]	None	--	54.6	0.0	1.0	9 600	255 000	18.2	54.6	47.8	190	0.032	0.185	96	+	0	0	7.3
[redacted]	F	[redacted]	None	--	64.1	0.0	0.0	13 400	405 000	22.4	52.6	37.2	117	0.044	0.177	101	+	0	0	2.5
[redacted]	M	[redacted]	None	--	87.8	0.0	0.2	11 000	250 000	25.1	87.7	47.4	72	0.053	0.261	111	+	0	0	12.3[a]
[redacted]	F	[redacted]	None	--	48.6	0.0	0.2	11 200	330 000	21.0	48.6	32.4	118	0.040	0.147	116	+	0	0	3.4
[redacted]	F	[redacted]	Y^{90}	2.3	62.8	2.3	2.3	10 500	224 000	22.0	62.8	19.8	120	0.030	0.153	132	+	0	0	16.7
Polycythemia vera Class IIa																				
[redacted]	M	[redacted]	None	--	48.6	0.1	0.6	8 900	210 000	18.9	43.0	34.0	80	0.036	0.217	68	+	0	0	--
[redacted]	M	[redacted]	None	--	41.1	0.1	0.8	8 800	835 000	13.7	34.4	45.3	88	0.026	0.130	81	+	0	0	--
[redacted]	M	[redacted]	None	--	40.5	5.8	3.0	20 800	770 000	18.5	36.0	37.9	86	0.022	0.124	96	+	0	0	--
[redacted]	M	[redacted]	None	--	70.8	0.7	0.7	14 800	620 000	16.7	41.5	41.4	89	0.050	0.320	41	+	0	0	--
[redacted]	F	[redacted]	None	--	43.2	0.0	3.0	9 900	625 000	19.8	43.2	39.0	210	0.067	0.269	51	+	0	0	--
[redacted]	M	[redacted]	None	--	42.8	3.1	0.0	8 050	360 000	17.6	39.6	42.0	170	0.025	0.151	86	+	0	0	--
[redacted]	M	[redacted]	None	--	34.2	3.9	3.0	11 700	380 000	18.5	31.7	37.5	113	0.015	0.051	62	+	0	0	--
[redacted]	M	[redacted]	None	--	65.2	0.5	0.5	11 500	310 000	19.0	64.1	33.8	82	0.084	0.452	39	+	0	0	--
[redacted]	M	[redacted]	None	--	61.0	0.0	0.5	10 700	675 000	18.9	41.0	33.2	149	0.030	0.182	71	+	0	0	--
[redacted]	M	[redacted]	splenectomy[c]	0.4	50.2	0.2	0.0	17 900	640 000	19.1	49.1	36.0	75	0.044	0.226	68	+	0	0	8.6[d]
[redacted]	M	[redacted]	P^{32}	0.8	46.0	0.0	11.0	18 000	300 000	21.4	46.0	26.9	200	0.050	0.218	68	+	0	0	16.4[a]
[redacted]	F	[redacted]	P^{32}	2.0	48.0	1.7	4.7	6 900	330 000	13.4	26.0	26.7	121	0.026	0.171	47	+	0	0	8.3[f]
[redacted]	M	[redacted]	P^{32}	0.5	41.1	3.2	3.2	6 600	280 000	20.2	49.7	36.0	140	0.051	0.276	58	+	0	0	13.2[b]
[redacted]	F	[redacted]	P^{32}	2.0	41.2	2.9	8.2	7 650	507 500	16.9	32.7	38.7	139	0.031	0.192	41	+	0	0	--
Polycythemia vera Class I-IIa, iron-deficient																				
[redacted]	M	[redacted]	None	--	44.5	0.0	0.0	7 400	275 000	20.0	44.5	34.3	50	0.028	0.154	75	+	0	0	--
[redacted]	F	[redacted]	None	--	59.2	0.1	0.2	10 150	300 000	20.0	59.2	38.5	35	0.035	0.243	44	+	0	0	--
[redacted]	F	[redacted]	None	--	65.6	0.0	0.2	9 700	255 000	20.6	65.6	30.7	46	0.045	0.244	60	+	0	0	--
[redacted]	M	[redacted]	None	--	38.3	0.0	10.2	8 700	435 000	13.4	38.3	55.6	62	0.039	0.244	58	+	0	0	--
[redacted]	F	[redacted]	None	--	52.2	0.0	0.0	6 380	360 000	17.1	52.2	45.4	60	0.054	0.258	60	+	0	0	--
[redacted]	F	[redacted]	None	--	62.2	0.1	0.2	9 300	210 000	19.1	40.5	34.9	36	0.020	0.132	60	+	0	0	--
[redacted]	M	[redacted]	None	--	40.0	0.0	0.3	11 050	775 000	15.0	35.4	36.4	20	0.023	0.127	57	+	0	0	3.1[b]
[redacted]	F	[redacted]	None	--	70.9	3.0	3.0	10 000	190 000	16.7	69.7	41.5	31	0.069	0.402	43	+	0	0	4.0
[redacted]	M	[redacted]	None	--	105.6	0.1	0.2	17 900	230 000	20.0	98.7	43.3	35	0.131	0.571	42	+	0	0	3.5
[redacted]	M	[redacted]	None	--	61.5	3.3	3.0	26 400	550 000	13.4	43.4	71.7	23	0.064	0.254	47	+	0	0	6.1[f]
[redacted]	M	[redacted]	None	--	71.8	0.0	0.5	19 550	180 000	18.2	71.8	36.7	41	0.030	0.200	33	+	0	0	0.8[f]
[redacted]	F	[redacted]	None	--	40.6	0.0	0.0	59 350	850 000	11.3	40.6	51.5	10	0.018	0.114	47	+	0	0	
[redacted]	F	[redacted]	splenectomy[c]	9.6	49.9	0.0	9.0	20 100	705 000	15.3	49.9	52.6	28	0.038	0.220	67	+	0	0	--
[redacted]	F	[redacted]	P^{32}	8.3	60.2	12.3	13.0	4 600	270 000	16.2	53.1	46.2	54	0.062	0.251	63	+	0	0	19.0[b]
[redacted]	M	[redacted]	P^{32}	1.5	66.3	7.3	10.8	16 700	410 000	16.1	26.3	40.5	56	0.041	0.286	29	+	0	0	13.2[b]
[redacted]	F	[redacted]	P^{32}	3.0	63.3	7.9	11.5	24 600	240 000	18.9	27.4	58.9	37	0.021	0.131	60	+	0	0	16.3[f]
[redacted]	M	[redacted]	P^{32}	5.6	50.0	9.0	9.0	21 000	955 000	16.7	40.8	52.0	19	0.034	0.195	59	+	0	0	--[e]

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Table I. Iron kinetics in polycythemia vera. (Continued)

R. G.	F	52	P^{32}	3.0	63.3	7.9	11.5	24 800	240 000	10.9	27.4	50.9	37	0.021	0.131	60	+	0	0	16.3[i]
J. M.	M	63	P^{32}	5.6	50.0	9.0	9.0	21 000	455 000	16.7	40.8	52.0	19	0.034	0.195	59	+	0	0	-- [h]
Polycythemia vera Class IIb																						
[redacted]	M	[redacted]	P^{32}	2.5	44.8	11.5	18.0	2 850	270 000	11.0	21.1	58.7	159	0.078	0.432	18	+	0	0	18.4[f]
	F		P^{32}	0.5	51.5	17.9	18.0	3 350	180 000	8.2	13.3	46.6	134	0.034	0.217	19	+	0	0	19.0[f]
Polycythemic vera Class III																						
[redacted]	M	[redacted]	None	--	52.3	16.8	13.0	26 300	260 000	13.6	35.0	51.9	23	0.039	0.233	26	+	+	+	17.0
	F		None	--	58.3	0.0	0.9	27 000	200 000	16.2	54.6	51.7	130	0.097	0.372	49	+	+	0	--
	F		None	--	8	--	18.0	14 500	210 000	9.3	33.2	78.7	48	0.105	0.528	20	+	+	+	21.8[e]
	F		None	---	45.8	0.0	10.0	204 000	300 000	13.1	45.6	71.4	59	0.061	0.381	37	0	+	+	12.0[e]
	F		None	--	46.0	0.0	0.0	9 500	206 800	12.3	49.5	68.0	111	0.081	0.476	26	+	+	0	-- [h]
	M		None	--	58.8	0.1	0.0	20 900	280 000	17.4	58.8	44.9	19	0.049	0.258	37	+	+	+	2.7
	F		P^{32}	2.1	49.6	19.0	25.0	12 700	200 000	10.3	17.5	46.3	59	0.028	0.184	32	+	+	+	29.0
	F		P^{32}	1.0	8	--	5.0	2 700	30 500	7.2	20.5	45.6	155	0.049	0.252	27	+	+	+	6.0
	F		P^{32}	10.0	8	--	6.8	35 000	106 000	7.6	30.1	87.9	203	0.118	0.735	8	+	+	+	0.6[e,h]
	M		P^{32}	2.6	81.6	10.0	10.0	6 800	60 000	10.0	28.0	69.7	49	0.085	0.467	18	0	+	+	--
	M		P^{32}	0.5	66.1	7.3	12.5	9 700	375 000	8.8	23.0	51.5	100	0.083	0.423	13	+	+	0	12.9[e]
	F		P^{32}	1.3	55.8	8.8	17.0	7 650	290 000	13.0	27.4	48.0	121	0.063	0.350	25	+	+	0	21.5[e]
	M		P^{32}	1.5	52.0	6.8	4.0	18 800	980 000	12.4	33.7	36.6	65	0.066	0.225	36	+	+	+	--
	M		P^{32}	0.7	61.9	0.8	6.0	44 000	760 000	6.4	37.8	113.2	5	0.033	0.196	40	+	+	+	7.6
	M		P^{32}	4.2	58.3	0.4	12.0	7 800	255 000	11.4	25.4	52.9	54	0.044	0.298	24	+	+	+	--
	M		spleen irrad.[c]	13.6	68.0	0.0	0.0	46 500	425 000	16.7	68.0	35.1	40	0.072	0.173	28	+	+	+	4.5
	M		P^{32}	0.8	8	--	3.6	22 700	140 000	8.9	30.7	66.2	80	0.092	0.258	31	0	+	+	5.0
	M		P^{32}	0.4	61.1	3.2	8.0	9 600	200 000	12.9	35.7	46.9	31	0.067	0.453	18	0	+	+	13.2
	M		P^{32}	0.6	68.7	5.1	7.0	13 500	88 000	8.3	27.1	71.1	97	0.177	0.964	8	+	+	+	7.8[e,h]
	M		P^{32}	0.8	73.3	8.3	9.0	25 700	190 000	13.6	49.6	53.7	44	0.061	0.201	67	0	+	+	10.8[e]
	F		P^{32}	0.6	48.0	0.0	2.5	72 500	210 000	12.0	36.5	41.1	17	0.040			+	+	+	3.1
	F		P^{32}	0.4	56.9	2.8	1.0	13 200	240 000	21.1	49.7	30.3	200	0.089	0.369	46	+	+	0	3.6[e]
	M		P^{32}	3.5	60.0	4.0	9.0	11 300	320 000	10.7	26.8	55.8	71	0.061	0.296	31	+	+	0	13.2[e]
	M		P^{32}	0.9	51.1	6.8	12.0	12 000	210 000	9.3	19.3	50.0	84	0.046	0.276	22	+	+	0	14.1[e]
				2.9	8	--	3.0	27 250	180 000	9.0	25.2	67.5	192	0.045	0.001	34	+	+	+	3.7[e]

a. Developed clinical findings of myeloid metaplasia (Class III) subsequent to study.

b. Developed clinical and iron kinetics findings of myeloid metaplasia (Class III) subsequent to study. For iron kinetics values at the time of repeat study see patient under results of Class III.

c. Not used in averaging in "treatment with P^{32}" group.

d. Developed terminal refractory anemia.

e. Myeloid metaplasia diagnosed by iron kinetics subsequently confirmed at post mortem examination.

f. Acute leukemia at post mortem examination.

g. Red cell volume determination not performed at the time hemoglobin and hematocrit elevated in these cases. However, in each case hemoglobin values in excess of 20.0 grams were recorded and leucocytosis and in some cases thrombocytosis was present and the patient had the clinical findings of polycythemia vera.

h. Myeloid metaplasia in spleen diagnosed by iron kinetics subsequently confirmed by splenectomy or splenic aspiration.

i. Development of myeloid metaplasia subsequent to study demonstrated at post mortem examination.

1160996

Table II. Averaged values of data from Table I (mean ±σ)

Measured parameter [normal values]	Class I		Class IIa		Class I - IIa c̄ Fe deficiency		Class IIb		Class III	
	No P^{32} Rx (9)	P^{32} Rx (1)	No P^{32} Rx (9)	P^{32} Rx (4)[b]	No P^{32} Rx (12)	P^{32} Rx (4)[b]	No P^{32} Rx (0)	P^{32} Rx (2)[c]	No P^{32} Rx (6)	P^{32} Rx (18)[b]
Age at time of Diagnosis (Dx)	53.1 ±13.6	56.0	39.1 ±18.2	53.8 ± 7.7	52.2 ±23.6	49.5 ± 9.7	—	45.0 56.0	56.5 ±12.7	55.9 ± 8.95
Maximum recorded RBC volume - ml/kg	64.4 ±14.5	62.8	47.5 ±11.6	49.1 ± 7.4	60.1 ±17.5	60.0 ±10.01	—	44.8 51.5	52.2 ± 5.7	60.3 ± 9.2
Time after Dx maximum RBC volume measured (yr)	1.2 ± 0.5	2.3	1.6 ± 2.0	2.0 ± 1.3	0.6 ± 1.3	9.6 ± 2.8	—	11.5 17.9	— —	6.0 ± 4.8
Time from Dx to death (yr)	8.6 ± 4.5	16.7	— —	9.5 ± 6.2	3.7 ± 1.8	— —	—	18.4 19.0	13.4 ± 7.1	10.7 ± 6.9
Time from Dx to Fe^{59} kinetics study (yr)	0.9 ± 1.23	2.3	1.3 ± 1.2	6.8 ± 3.0	1.5 ± 2.8	11.4 ± 2.3	—	18.0 18.9	7.0 ± 7.1	8.5 ± 5.7
WBC - (No./mm^3) [5 000 - 10 000]	18 000 ±16 000	10 500	11 683 ± 3 746	9 788 ±4 757	16 383 ±14 128	16 775 ± 7 591	—	2 850 3 350	50 366 ±68 984	19 605 ±16 531
Platelets - (No./mm^3) [250 000 - 500 000]	305 000 ±55 000	224 000	531 666 ±208 415	368 750 ±159 513	384 167 ±217 865	468 750 ±287 967	—	270 000 180 000	242 830 ±39 062	268 583 ±232 028
Hemoglobin (g/100 ml) [14.0 - 16.0]	21.3 ± 2.2	22.0	17.7 ± 1.8	18.0 ± 3.1	17.1 ± 3.0	15.0 ± 2.4	—	11.0 8.2	13.7 ± 2.6	10.7 ± 3.3
RBC volume (ml/kg) [24 - 33]	60.8 ±14.3	62.8	41.6 ± 8.8	38.6 ± 9.6	55.0 ±18.0	36.9 ±11.0	—	21.1 13.3	46.3 ± 9.3	30.2 ± 8.8
Plasma volume (ml/kg) [32 - 45]	35.8 ± 7.1	19.8	38.2 ± 3.9	34.6 ± 4.5	43.4 ±11.0	47.4 ± 4.5	—	58.7 46.6	61.1 ±12.2	57.5 ±19.1
Serum iron (µg %) [70 - 170]	128 ±48	120	119 ±44	150 ±30	37 ±15	42 ±15	—	159 134	65 ±42	90 ±60
Plasma iron turnover - mg/kg/hr - [0.059 ± 0.004][a]	0.040 ± 0.016	0.030	0.039 ± 0.022	0.040 ± 0.011	0.046 ± 0.03	0.040 ± 0.015	—	0.078 0.034	0.072 ± 0.024	0.069 ± 0.035
Calculated Hgb Synthesis - g/kg/day [0.089 ± 0.014][a]	0.172 ± 0.046	0.153	0.211 0.114	0.214 ± 0.040	0.244 ± 0.124	0.216 ± 0.059	—	0.432 0.217	0.375 ± 0.106	0.355 ± 0.209
Mean erythron life span (days) [117 ± 7.5][a]	118 ±13.6	132	66.1 ±18.7	54 ±10.4	52.2 ±11.0	52.8 ±13.8	—	18 19	34.7 ±11.6	28.2 ±14.2

a. Normal values derived from data on 13 normal subjects.

b. Patients treated by splenectomy or splenic radiation are not included in averaging values.

c. Values of each of the two patients in this class are listed separately.

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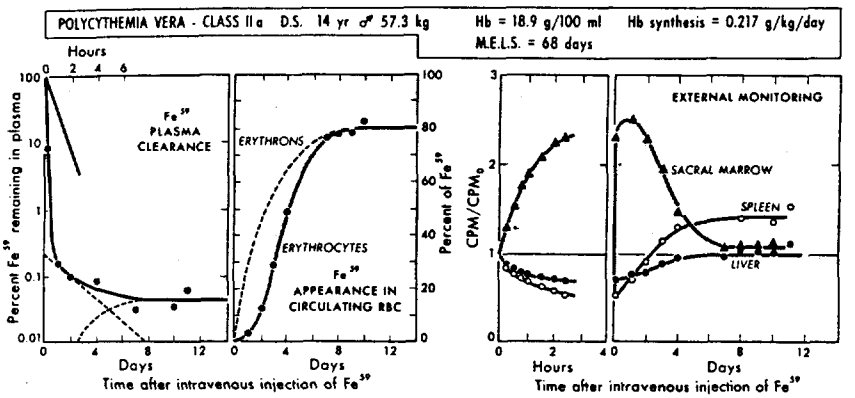

Figure 2. In polycythemia Class IIa ^{59}Fe kinetics, plasma-iron turnover is increased with the plasma-radioiron curve reaching an asymptote at the time maximum incorporation of ^{59}Fe in RBC is reached. If hemolysis is marked, the maximum incorporation of ^{59}Fe in RBC may be below the normal range. External monitoring over sacral bone marrow and liver have normal patterns, but the spleen usually shows a secondary rise above baseline indicating splenic sequestration of red cells. By correcting the plasma-radioiron curve for extramedullary hemolytic feedback (see Materials and Methods section) a curve having two or three exponential components is obtained. Analysis of plasma-radioiron data thus corrected for hemolytic feedback yields increased values for rate of hemoglobin formation and shortened mean erythron life span.
MUB-6191

prior to release into the circulating blood (intramedullary hemolysis). Figure 2 is a typical example of the iron-kinetics pattern designated polycythemia vera Class IIa. In this class there is again rapid accumulation of iron in the sacral marrow with subsequent prompt, virtually complete release as the iron leaves the marrow in circulating red-blood cells. Generally, there is a secondary rise in the counting rate over the spleen indicating splenic sequestration of red blood cells. The shape of the curve representing net ^{59}Fe incorporation into circulating RBC may be within normal limits, but when there is considerable shortening of red-cell survival the maximum net incorporation of ^{59}Fe into circulating RBC is depressed below normal values. Plasma-iron turnover is increased above normal values (class average of nine patients not given previous radiation therapy in Table 2 is 0.039 mg/kg/hr $\pm \sigma$ = 0.022). The kinetic analysis of plasma-radioiron-clearance curves demonstrates increased hemoglobin synthesis (same class average in Table 2 is 0.211 g/kg/d $\pm \sigma$ = 0.114). Shortened red-cell life span is seen (same class average = 66.1 days $\pm \sigma$ = 18.7), and the plasma radioiron becomes constant between six to ten days, in keeping with hemolytic feedback of radioiron into the plasma from red cells destroyed in the spleen. This iron-kinetic pattern is similar to that

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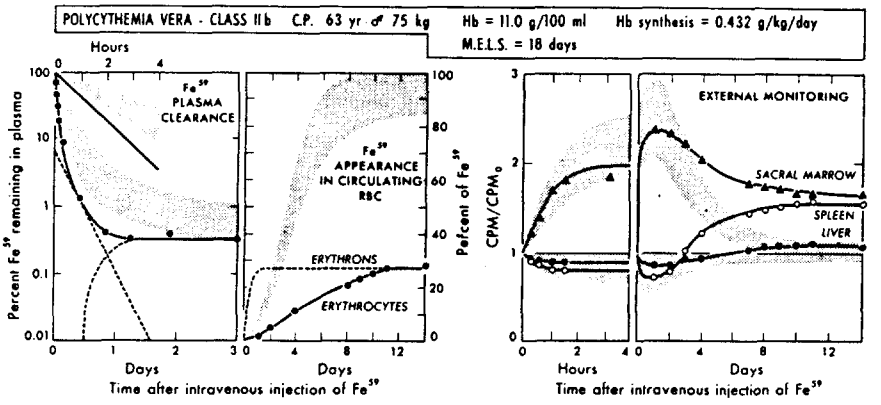

Figure 3. In polycythemis Class IIb ^{59}Fe kinetics, plasma-iron turnover is increased with the plasma-radioiron curve reaching an asymptote much earlier than the time of maximum incorporation of ^{59}Fe into circulating RBC. The appearance of radioiron in circulating RBC is markedly delayed and mean erythron hemoglobinization time is markedly prolonged (7). The external monitoring curve over the sacral bone marrow fails to return to baseline with net retention of radioiron, and the spleen curve frequently shows a variable secondary rise indicating splenic sequestration of RBC. This pattern is identical to that seen in the megaloblastic anemias and Thalassemia major (8), indicating intramedullary hemolysis of erythrocytes and their precursors. When the plasma-radioiron curves are corrected for intramedullary hemolysis (see Materials and Methods section) and the resultant curves are analyzed, hemoglobin synthesis is found to be markedly increased and mean erythron life span is markedly shortened.
MUB-6192

seen in congenital spherocytic anemia and a variety of acquired hemolytic anemias characterized by extramedullary hemolysis (8).

In Class IIb there is markedly shortened red-cell survival, which is predominantly the result of intramedullary hemolysis. This has been studied in only two patients, both of whom had been treated with ^{32}P and were noted to be rapidly deteriorating 18 years following the initial diagnosis of polycythemia vera. In both of these cases anemia was rapidly progressive and hepatosplenomegaly was not marked. External monitoring, as shown in Fig. 3, demonstrates a rapid initial accumulation of iron in the sacral marrow with markedly delayed and incomplete secondary release. Plasma-iron turnover is increased (in case shown in Fig. 3 was 0.078 mg/kg/hr). There is no evidence of extramedullary erythropoiesis in liver or spleen. Kinetic analysis of plasma-radioiron data of the patient shown in Fig. 3 revealed a markedly increased hemoglobin synthesis of 0.432 mg/kg/d with a markedly shortened RBC

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life span of 18 days. Maximum incorporation of radioiron into circulating red blood cells is delayed and markedly decreased. A very early hemolytic feedback of iron to plasma is observed in the plasma-radioiron curve. The mean effective erythron-hemoglobinization time (7) is markedly prolonged, correlating with the incomplete release of radioiron from the sacral bone marrow. This pattern is similar to that seen with refractory anemia with erythroid hyperplasia, thalassemia major and the megaloblastic anemias, and is interpreted as representing severe intramedullary hemolysis (8). This is substantiated by the finding of marked erythroid hyperplasia in the bone marrow without an increase of circulating reticulocytes in these patients at the time of kinetic analysis.

The iron-kinetics pattern designated as Class III is characterized by extramedullary hemopoiesis in spleen and sometimes in liver. An example of this class can be seen in Fig. 4. There is an initial accumulation of iron in the spleen and sometimes in the liver with subsequent release as the iron leaves these organs in newly formed red cells. There may or may not be evidence of red-cell production within the bone marrow (see Table 1). The incomplete net release of radioiron from the spleen is interpreted as representing splenic sequestration in addition to splenic production of red blood cells. Plasma-iron turnover is generally greater than that seen in Classes I and IIa (Class III average of six patients not given previous radiation therapy in Table 2 was 0.072 mg/kg/hr $\pm \sigma = 0.024$). Kinetic analysis of the plasma-radioiron data reveals further increase in hemoglobin synthesis (same class average in Table 2 = 0.375 g/kg/d $\pm \sigma$ = 0.106) and a further shortening of the mean erythron life span, (same class average in Table 2 = 34.7 days $\pm \sigma = 11.6$). There is marked early hemolytic feedback of iron into the plasma as seen in the plasma-radioiron data. The appearance of ^{59}Fe in circulating RBC is early and rapid, but the maximum net incorporation of radioiron into circulating red cells is distinctly reduced as a result of the destruction of red cells, primarily within the spleen.

In addition to the above mentioned groups there are those polycythemic patients without evidence of myeloid metaplasia or intramedullary hemolysis who were iron deficient at the time of the study. Because of the shortened mean erythron life span and variable splenic sequestration of red cells seen in iron deficiency per se (8), these patients could not be differentially classified between Class I and Class IIa. Consequently the iron-kinetics values obtained for members of this group were the result of the abnormalities of iron deficiency superimposed upon the values of Classes I and IIa (see Tables 1 and 2).

EFFECT OF RADIATION THERAPY ON PATTERNS OF ERYTHROPOIESIS Tables 1 and 2 summarize the results of individual iron-kinetic studies correlated with clinical and pathologic features of each of the 64 patients studied. The results were organized according to the classes defined above. In each class the patients who had received no radiation therapy (^{32}P, ^{90}Y, X ray) prior to the time of the iron-kinetic study are listed separately from those patients who had received previous radiation therapy. In all cases, those patients without radiation therapy had received only venesection. In the group that had radiation therapy prior to the study, only those patients are included who had their last course of radiation

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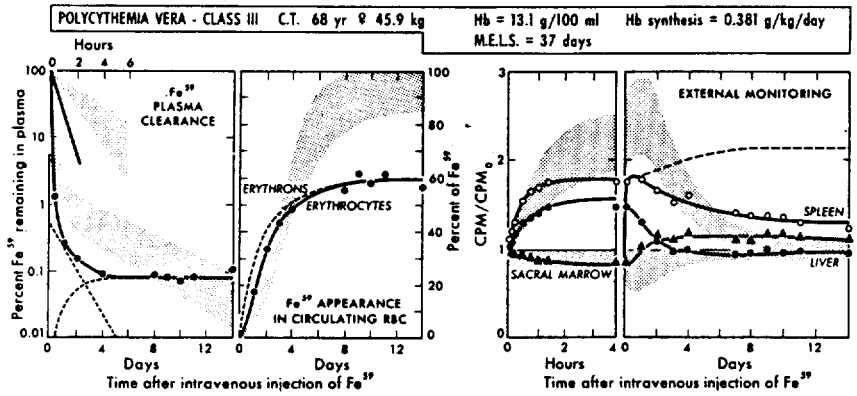

Figure 4. In polycythemia Class III ^{59}Fe kinetics, plasma-iron turnover is increased with the plasma-radioiron curve reaching an asymptote at the time maximum incorporation of radioiron into circulating RBC is reached. ^{59}Fe rapidly appears in circulating RBC, but the maximum incorporation of radioiron into circulating RBC is subnormal because of early hemolysis. Erythropoiesis may or may not be present in bone marrow or liver (see Table 1). The external monitoring curve over a site in which erythropoiesis is present shows an initial rise in counting rate with a subsequent fall concurrent with the appearance of radioiron in circulating erythrocytes. Splenic sequestration of RBC is frequently present resulting in incomplete loss of radioiron from the spleen with failure of the spleen curve to return to baseline values. When splenic sequestration of RBC exceeds splenic erythropoiesis, a spleen curve is obtained similar to the broken line shown on the right hand portion of this figure, in which the magnitude of the secondary rise in counting rate over the spleen exceeds the initial maximum counting rate seen at the end of the first 24 hr. Correction of the plasma-radioiron curve for "extramedullary" type hemolysis and analysis of the resulting curves yields increased rates of hemoglobin synthesis and decreased mean erythron lifespans (Tables 1 and 2).

MUB-6193

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therapy more than five months prior to the iron-kinetics study, eliminating those patients in whom the acute affects of radiation would affect red-cell kinetics.

At the time of ^{59}Fe-kinetics study, 10 patients were in Class I (nine untreated by radiation); 14 patients were in Class IIa (nine untreated by radiation); 25 patients were in Class III (six untreated by radiation); two patients were in Class IIb and 17 patients were in iron-deficiency-combined Classes II and IIa (12 patients untreated by radiation). Four of these patients appear in two groups because of change in erythropoietic class determined by repeat iron kinetics study (see Table 3). Within each group the results obtained on patients who had received prior radiation therapy were comparable to those obtained on patients who had not received radiation therapy prior to the time of the study. However, the mean values for red-cell volume and body hemoglobin in treated patients in Classes IIa and III tend to be somewhat lower than in untreated patients in these categories.

INCIDENCE OF IRON DEFICIENCY Of 43 polycythemic patients without myeloid metaplasia (Classes I and II with and without iron deficiency), 17 were found to be iron deficient at the time of the study (serum iron < 70 μg %), and of 25 patients having myeloid metaplasia (Class III) 13 patients were found to be iron deficient at the time of the study (Table 1). These results demonstrate the very high frequency with which iron deficiency (resulting from either spontaneous blood loss or phlebotomy) complicates the course of polycythemia vera. Since iron deficiency *per se* results in decrease in mean erythron life span and increased rate of hemoglobin synthesis, this factor contributes significantly to the abnormalities in erythropoiesis seen in this disease (8).

IRON-KINETICS DIAGNOSIS OF MYELOID METAPLASIA The iron-kinetics evidence for localization of erythropoiesis in a given area depends upon the demonstration of an initial rapid increase in counting rate over the given area with subsequent decrease of counting rate over these sites, simultaneous with the corresponding appearance of radioiron in circulating erythrocytes. However, under the circumstances in which splenic sequestration of red cells exceeds splenic erythropoiesis, (see legend Fig. 4) a delayed secondary rise in the counting rate over the spleen may obliterate evidence of the initial release of iron from the spleen.

In each patient in whom the iron kinetics study was interpreted to demonstrate myeloid metaplasia, the finding was confirmed clinically, (i.e., clinical evidence included the combinations of findings of marked hepatosplenomegaly, marked anisocytosis, poikilocytosis, circulating nucleated red cells, platelet and white-cell abnormalities). Three patients, classified in Class III by iron kinetics, had the diagnosis of myeloid metaplasia confirmed by splenic aspiration or splenectomy. In 11 patients the iron-kinetics diagnosis of myeloid metaplasia was confirmed at postmortem examination (two of which had previous confirmation by splenic aspiration or splenectomy). In two patients in whom a secondary delayed rise in splenic counting rate was noted, suggesting splenic sequestration and destruction of red cells in excess of splenic erythropoiesis, subsequent splenectomy resulted in cessation of the need for supportive transfusions and increased hemoglobin concentration (patients [redacted] and [redacted] Table 1).

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HEPATIC ERYTHROPOIESIS AND IRON DEFICIENCY In all seven cases listed in Table 1 in which myeloid metaplasia was present in the spleen but not in the liver, the serum iron was within normal limits at the time of the study (Table 1). (There was one additional patient, not included in Tables 1 and 2, in whom only a partial iron-kinetics study was performed, erythropoiesis was present only in the spleen and the serum iron was low -- [redacted], Table 3). In 13 out of 18 cases where myeloid metaplasia was present in the liver as well as the spleen the patients were iron deficient at the time of study (Table 1). In the remaining five cases where erythropoiesis was present in the liver but the serum iron was not depressed, there was evidence for excessive splenic sequestration and destruction of red cells in the spleen with an average mean erythron life span = 21.6 d. In one case ([redacted] Table 3) an initial iron-kinetic study revealed erythropoiesis in spleen but not in the liver and a repeat iron-kinetics study two years later revealed erythropoiesis in both liver and spleen. In the second study there was marked splenic sequestration of red cells with a marked increase in serum-iron turnover and increased hemoglobin synthesis with markedly reduced mean erythron life span. These results suggest that as net hemoglobin synthesis increases, myeloid metaplasia first occurs in the spleen and when further reduction in mean erythron life span and further increase in hemoglobin synthesis occur, either because of superimposition of iron deficiency or marked shortening of RBC survival with splenic sequestration of red cells, myelopoiesis may then be seen in the liver.

BLOOD VOLUME CHANGES The blood volume (sum of independently measured red-cell volume and plasma volume) is increased in all classes of polycythemia studied (11). The greatest increase is seen in Class III (mean values for blood volume for the various classes from Table 2 for patients not given radiation therapy prior to study are in Class I = 96.6 ml/kg, Class IIa = 79.8 ml/kg, Class IIb = 69.9 ml and Class III = 107.4 ml/kg). The increase in blood volume in the members of Class III was roughly correlated with the degree of hepatosplenomegaly present and was associated with a markedly expanded plasma volume. Because of the markedly increased blood volume the red-cell volume (ml RBC/kg body wt.) was frequently found to be in the normal range though the hemoglobin concentration indicated an anemia.

EVOLUTION OF ERYTHROPOIETIC PATTERNS Two patients initially found to be in Class I, and one patient initially found to be in Class IIa, as well as one patient initially found to be in iron deficiency Classes I and IIa, during their subsequent course developed myeloid metaplasia as determined by clinical criteria alone (see above). One patient initially found to be in Class IIa and two patients initially in iron-deficiency Classes I and IIa during their subsequent course were found to develop myeloid metaplasia as determined by histologic examination at postmortem examination in addition to clinical criteria (see Table 1). In one patient initially in Class I and one patient initially in Class IIa, as well as three patients initially in Class IIa, as well as three patients initially in iron deficiency Classes I and IIa, the transition to Class III (myeloid metaplasia) was initially demonstrated by repeat iron-kinetics studies in addition to clinical criteria (see Table 3). The two patients found to be in Class IIb at the time of iron kinetics had no evidence of preceding myeloid metaplasia and thus would have been classified in Classes I and IIa earlier in their course. The time from diagnosis to

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Table III. Hematologic values of patients at the time of repeat iron kinetics studies

nt	Class	Time from Dx to study (years)	Last Radiation Rx prior to study (years)	WBC (No./mm^3)	Platelets (No./mm^3)	Hgb (g %)	RBC Vol. (ml/kg)	Plasma Vol. (ml/kg)	Serum Fe (μg %)	Plasma Fe turnover (mg/kg/hr)	Calculated Hb synthesis g/kg/day	Net mean Erythron life span (days)	Erythropoietic site Marrow	Spleen	Liver
	I	0.4	None	25 5000	340 000	19.6	52.6	35.2	119	0.037	0.128	127	+	0	0
	III	12.0	4.2	7 800	255 000	11.4	25.4	52.9	54	0.044	0.298	24	+	+	+
	II_a	3.2	0.5	6 600	280 000	20.2	49.7	36.0	140	0.051	0.276	58	+	0	0
	III	8.0	0.4	9 600	200 000	12.9	35.7	46.9	31	0.067	0.453	18	0	+	+
	III	5.0	0.3	8 200	220 000	14.2	66.7	68.3	48	0.124	0.751	23	+	+	0
	III	7.0	0.6	13 500	88 000	8.3	27.1	71.1	97	0.177	0.964	8	+	+	+
	III	0.9	None	27 000	200 000	16.2	54.6	51.7	130	0.097	0.372	49	+	+	0
	III	13.5	6.0	13 500	9 000	13.0	36.3	58.7	76	0.094	0.545	22	+	+	0
	I-II_a c̄ Fe defic.	10.0	1.5	16 700	410 000	16.1	26.3	40.5	56	0.041	0.286	29	+	0	0
	III	12.5	0.5	9 700	375 000	8.8	23.0	51.5	100	0.083	0.425	13	+	+	0
	I-II_a c̄ Fe defic.	0.3	None	11 850	775 000	15.0	35.4	36.4	20	0.023	0.127	57	+	0	0
	III	2.4	0.6	72 500	210 000	12.0	36.1	41.4	17	0.040	--	--	+	+	+
	I-II_a c̄ Fe defic.	13.0	0.3	4 600	270 000	16.2	53.1	46.2	54	0.062	0.251	63	+	0	0
	III	18.0	1.8	14 200	220 000	12.1	--	--	28	0.065	--	--	+	+	0

artial Study -- radioiron clearance from plasma was not obtained after the first day, making calculation of Hb synthesis impossible. This study not included
data in Tables I and II.

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study of patients in Classes IIb and III (mean times for untreated groups being 18.0 years and 7.0 years respectively -- Table 2) is significantly longer than patients in Classes I and IIa, (mean time for untreated groups being 0.9 years and 1.3 years respectively -- Table 2), suggesting that patients in the former classes had had their disease for a longer period of time than patients in the latter classes. Many patients who were found to be in Class III at the time of the iron-kinetics study previously had clinical histories and hematological findings that suggested that earlier in their course myeloid metaplasia had not been present. These results indicate that patients in Classes I and IIa are in earlier phases of their disease than patients in Classes IIb and III. As time passes many patients in Classes I and IIa may progress into Class III (myeloid metaplasia) or a few may progress into Class IIb (refractory anemia with erythroid hyperplasia and intramedullary hemolysis).

In all patients in whom repeat iron-kinetic studies were performed during the course of their disease, calculated hemoglobin synthesis increased simultaneous with a shortening of mean erythron life span and decreasing hemoglobin-concentration values during their second study (see Table 3). This same pattern is seen when plasma-iron turnover and hemoglobin synthesis are related to the mean time after diagnosis of untreated patients in Classes I, IIa and III, as seen in Fig. 5. An even greater rate of hemoglobin synthesis and shortening of mean erythron life span is seen in the two patients in Class IIb (time from diagnosis to study = 18 years).

As can be seen from Tables 1, 2 and 3, and in Fig. 5, there is a rough direct correlation between hemoglobin synthesis and white-blood-cell count (3, 11). Since, at least in part, the white blood count is a reflection of white-cell-production rate one may postulate that during the course of polycythemia vera the rate of white-cell production increases in a fashion similar to the rate of red-cell production. The concept that white-cell production is increased in certain patients with polycythemia vera has been previously postulated (12) and gains some support from published work (13).

As can be seen from Tables 1 and 2, patients in Class III tend to have lower platelet counts than patients in Classes I and IIa. Since, following their release from the bone marrow, the platelets (similar to the red cells) spend the major portion of their functional life in the blood, the finding that their concentration, similar to the hemoglobin concentration, tends to be decreased in patients in Class III suggests that, as with red cells, the functional survival of platelets is decreased in patients in this class. The concept that platelet survival parallels RBC survival in this disease gains support from ^{32}DFP platelet studies which we performed in two polycythemic patients. In both patients platelet survival was shortened roughly in proportion to shortening of RBC survival. In one patient in erythropoietic Class IIa, the mean platelet survival was 5.2 days, and in the other patient in erythropoietic Class III the mean platelet survival was 2.4 days (normal 8-12 days).

DISCUSSION

In polycythemia-vera there is a shortening of red-cell life span as the disease progresses, which is incompletely compensated by an increase in red-cell synthesis, resulting

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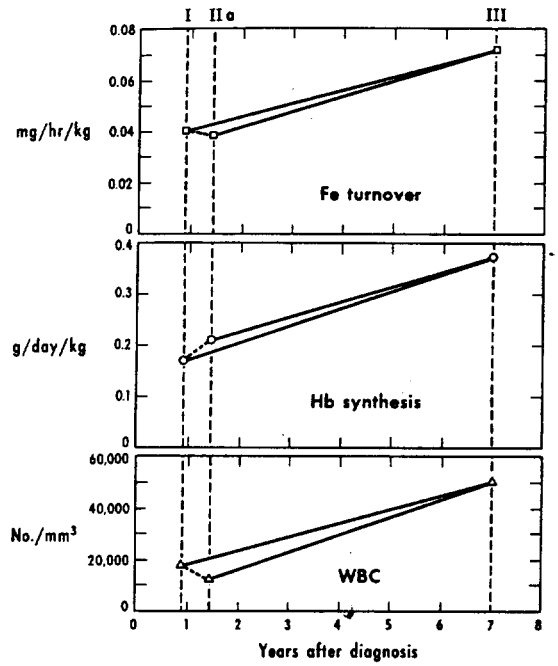

Figure 5. Mean values for plasma-iron turnover, hemoglobin synthesis and white-blood-cell (WBC) count of patients not receiving radiation therapy prior to study in Classes I, IIa, and III are plotted as a function of time from diagnosis to study.
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in a progressive decrease of red-cell volume. This occurs whether or not X ray or radioisotopes are used in therapy and appears to be a result of the natural history of the disease. Splenic sequestration of red cells is correlated with this shortening of red-cell survival. As the disease progresses and the rate of hemoglobin synthesis increases, myeloid metaplasia may appear at first in the spleen. The close association of hepatic myeloid metaplasia with marked splenic sequestration of red cells and iron deficiency suggests that hepatic erythropoiesis occurs when red-cell survival is markedly shortened with marked splenic destruction or with the additional complication of further decrease of red-cell survival due to iron deficiency. It is possible that iron deficiency leads to nonspecific selective advantage of the liver for erythropoiesis relative to the non-iron deficient state (e.g., large plasma-iron perfusion rate of liver).

In certain cases an abrupt marked shortening in mean erythron life span may occur prior to the development of myeloid metaplasia, as a result of marked intramedullary destruction of red cells (Class IIb). The development of this situation appears ominous since in both of these cases with prior ^{32}P therapy, death ensued within a year of the iron-kinetic study. Patients without myeloid metaplasia who have a terminal severe anemia with marrow showing erythroid hyperplasia have been described by others in the course of polycythemia vera not

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treated by radiation therapy (6). Whether the situation which we have described as polycythemia vera Class IIb is identical with the erythroleukemia-type picture described by others or is a state preceding the development of acute leukemia is open to speculation.

It is suggested that the pattern of evolution of granulocyte and platelet kinetics in polycythemia vera may be similar to that of the red cells as discussed above. Since as with red cells, the platelets spend the major portion of their functional life span in the blood, the tendency for lowered values of both platelet and hemoglobin concentration in Class-III patients suggests that during the course of polycythemia vera the platelets, as well as the red cells, have decreasing functional survival with incompletely compensatory increased production. Two studies of platelet survival in this disease are consistent with this hypothesis. Similarly, since the concentration of white cells in the blood appears to be relatively independent of the "functional survival" of the white cell, the tendency towards increasing white-cell counts as the duration of the disease increases may primarily reflect increasing rates of white-cell production. If these speculations are correct, then the course of changes in the red cells, white cells and platelets in polycythemia vera, can be described in terms of the progressive emergence of clones of hematopoietic cells which have selective advantage for reproduction but have altered functional capacity or shortened functional survival. Whether the emergence of such altered cell clones may be caused by mutational events, replication error, viral-induced cell changes, or in response to adnormal humoral stimuli involves second order speculation at the present time.

The results of this study have two therapeutic implications in the treatment of myeloid metaplasia (Class III) appearing in the course of polycythemia vera. The frequent finding of iron deficiency in these patients suggests usefulness of iron therapy in the treatment of their anemia. In those patients in whom splenic sequestration of red cells appears to exceed splenic erythropoiesis (as evidenced by a secondary increase in amplitude of external monitoring counting rate over the spleen) splenectomy should be of aid in reducing the anemia.

The pattern of evolution of erythropoiesis described above applies to patients whether or not they received radiation therapy or phlebotomy alone. The present series is too small for determining whether or not there is any statistical difference in the rate of evolution of erythropoietic patterns in this disease in patients treated with radiation versus those treated by phlebotomy alone.

CONCLUSIONS

Patients with polycythemia vera may be classified according to their erythropoietic pattern. Erythropoiesis is abnormally increased in all classes. Class I is characterized by normal red-cell life span. Class II is characterized by shortened red-cell life span: in Class IIa the shortened red-cell survival is related to splenic sequestration of RBC; in Class IIb the markedly shortened red-cell survival is predominantly related to intramedullary hemolysis. Class III is characterized by extramedullary erythropoiesis. Patients in Classes I and IIa are in relatively earlier phases of their disease and frequently are found to develop red-cell kinetics of Class III as their disease progresses. Conversely, patients in Classes

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IIb and III are generally late in the course of their disease and have previous hematologic findings that suggest that they originally had the red-cell kinetic patterns of Classes I and IIa.

As the duration of their disease increases, patients with polycythemia vera generally have progressive shortening of red-cell life span which is incompletely compensated by progressive increase in red-cell production with resultant progressive decrease in circulating red-cell volume. However, total blood volume is maintained elevated as plasma volume increases. Similar changes may occur in white-cell and platelet production and in functional survival in this disease. These changes occur whether or not the patient is given radiation therapy. It is suggested that the natural history of the disease may be characterized by progressive emergence of hematopoietic cell clones that have a selective advantage for reproduction associated with altered functional survival.

The results suggest the potential usefulness of iron or splenectomy in polycythemic patients with myeloid metaplasia (Class III) and anemia dependent upon the presence of the frequent finding of iron deficiency or the occasional finding of splenic sequestration of red cells in excess of splenic erythropoiesis.

ACKNOWLEDGMENTS

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