RADIOACTIVE IRON METABOLISM AND ERYTHROCYTE SURVIVAL STUDIES OF THE MECHANISM OF THE ANEMIA ASSOCIATED WITH RHEUMATOID ARTHRITIS [1]

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Anemia of moderate severity occurs frequently in rheumatoid arthritis patients (1). These patients show abnormalities of iron metabolism such as low serum iron concentration and rapid loss of injected iron from the plasma (1–4). Abnormalities of red cell survival have been described (5–7), while studies of heme pigment excretion products have not shown evidence of increased hemoglobin catabolism (8). The role of these disturbances in the genesis of the anemia in arthritic patients is not clearly understood.

The present study was undertaken to define the role of changes in rates of red cell production and destruction in the pathogenesis of the anemia occurring in rheumatoid arthritis patients, and to clarify the effect of disturbances of iron metabolism on red cell production in these patients. An abstract of this work has been published previously (9).

MATERIALS AND METHODS

A. Clinical material

1. Patients. The patients studied had active rheumatoid arthritis and were hospitalized for at least the first three weeks of the study. Cases were selected without regard for the presence or degree of anemia. Those with evidence of active bleeding or past history of significant blood loss were excluded from the study. Study cases received only conservative therapy during the period of this investigation, consisting of bed rest, physiotherapy, and salicylates. None of the study cases were known to have received previous blood transfusions.

2. Controls. The control subjects were normal healthy adults who worked full time and had no past or present evidence of chronic disease or of blood loss. These subjects were selected in an age group comparable to that of patients studied. Controls were not screened for normal serum iron or hemoglobin levels. Healthy male medical students served as blood donors and recipients of blood transfusions from arthritis patients for red cell survival studies.

B. Plan of study

Patients were fasted from 8 P.M. the evening preceding the study. From 8:30 to 9:30 A.M. blood was withdrawn for routine hematology and for serum iron determination. Immediately following this, Evans' blue dye and plasma-bound radioactive iron were successively injected intravenously using gravimetrically calibrated syringes (± 0.3 per cent). Samples of venous blood were taken from the opposite arm through an indwelling needle at 10-minute intervals for the first 30 minutes and at 30-minute intervals during the subsequent 3 to 5 hours. Over the next 14 to 21 days samples of venous blood were taken for measurement of red cell uptake of the injected radioiron. Stools were collected for measurement of fecal urobilinogen excretion. After completion of these studies, patients found suitable for red cell survival studies were given a blood transfusion and had samples of venous blood removed every 7 to 14 days during the following 120 to 140 days.

C. Radioiron studies

1. Isotope. Fe-59 of high specific activity (1 to 5 millicuries per milligram) was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, in the form of ferric chloride. The isotope was converted chemically to ferric ammonium citrate and the pH of the solution adjusted to 6.8 to 7.0. The latter was then diluted to an appropriate volume and sterilized by autoclaving.

2. Radioactivity detection techniques. All radioactivity assays were performed with gamma scintillation

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counting equipment, using thallium-activated, sodium iodide crystals. Liquid samples were counted in a 1-inch thick, well-type crystal. The counting efficiency was approximately 4 to 5 per cent. All samples were counted under conditions of constant geometry and sufficient counts recorded to give a counting error of less than 2 per cent. Standard iron solutions were counted in alternation with samples to correct for instrument variation and to eliminate the necessity for decay correction. External body monitoring was done using a 1-inch by 1-inch sodium iodide crystal housed in a lead-shielded portable probe which could be placed in contact with the skin over various organs (*e.g.*, spleen, liver). Activity was expressed as "counts per minute."

3. Plasma Fe-59 removal rate (clearance). Heparinized plasma, freshly drawn from a normal donor, was used as a source of iron-binding globulin. One-tenth to 0.3 ml. of the Fe-59 ammonium citrate was added to 12 to 15 ml. of plasma. Less than one microgram of inorganic iron was added to each ml. of plasma and the plasma was then incubated at 37° C. for 30 minutes to insure complete binding of the added iron to available iron binding protein. The total radioactivity injected ranged from 5 to 10 microcuries, and the total quantity of inorganic iron injected ranged from 3 to 12 micrograms. Standards were prepared by appropriate saline dilution of an aliquot of the original Fe-59 transferrin solution used for injection. Plasma samples obtained during the 3 to 5 hours after injection of the Fe-59 labelled plasma were assayed for radioactivity. The rate of removal of Fe-59 from the plasma was computed from these data using two different models (Figure 1).

Model A: This computation was done by the method of Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence (10). In this model, the Fe-59 clearance is assumed to be an exponential function of time. A straight line was fitted by the method of least squares to the data (y = log Fe-59 activity [net counts per minute], X = time [hours]). The Fe-59 space was determined from the computed value of this line at zero time. The time required for the removal of 50 per cent of the Fe-59 activity was computed from these data.

Model B: In many instances these values when plotted on semi-log paper showed what appeared to be a slight but definite curvature. Thus a second degree curve of the form (log counts $= a + bt + ct^2$) was fitted by the method of least squares. The half-time of Fe-59 removal and the Fe-59 space were computed from a tangent to this curve at time zero by the method of Dornhorst (11).

4. Plasma iron turnover rate. This was calculated by the method of Huff and his co-workers (10), using the following formula: Ln 2/half time plasma Fe-59 clearance [hours]) × Fe-59 space [ml.] × serum iron concentration [mgm. per ml.] × 24 = plasma iron turnover rate [mgm. per day]).

5. Red cell uptake of Fe-59. Four ml. of whole blood was hemolyzed with saponin and assayed for radioactivity. A hematocrit reading was obtained on an aliquot. The activity of each sample expressed as counts per minute per ml. of packed red cells, multiplied by the red cell mass of the subject, gave the total activity present in the red cell mass. This value, divided by the injected activity, gave the per cent red cell uptake of injected Fe-59.

6. Erythrocyte iron turnover rate. This was calculated by multiplying plasma iron turnover rate (No. 4) by the maximum per cent red cell Fe-59 uptake (No. 5), and expressed as mgm. per day.

7. External body monitoring. Body surveys were made at 30 to 60-minute intervals on the day of injection of the Fe-59 and at 1 to 3-day intervals thereafter. The detecting probe was placed directly on the skin over indelibly marked organ sites. The gross counting rate was corrected by subtracting the counting rate over the thigh to give the net counting rate at each organ site. An Fe-59 standard was counted each time a body survey was done and the counts were then corrected for decay to the activity at Day One.

8. The significance of the difference between means (p) was computed by the method of Aspin and Welch (12).

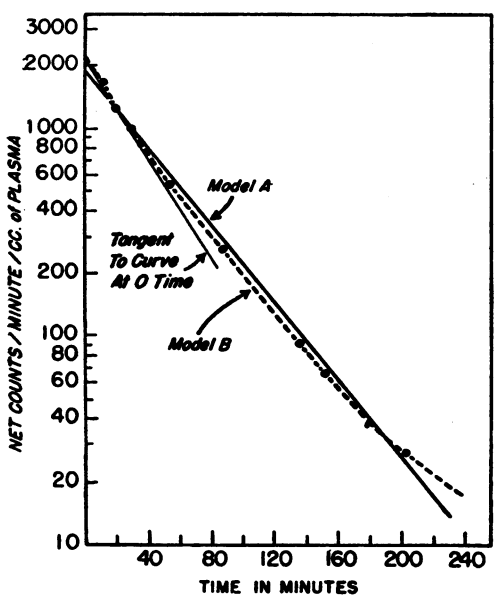

FIG. 1. GRAPHIC PRESENTATION OF THE TWO MODELS USED FOR COMPUTATION OF THE RATE OF REMOVAL OF RADIOIRON FROM THE PLASMA

D. Serum iron concentration

Serum iron was determined by the method of Kitzes, Elvehjem, and Schuette (13), using an Evelyn photoelectric colorimeter and orthophenanthrolene as an indicator. Samples were tested in duplicate and any showing hemolysis were discarded.

E. Plasma, red cell and total blood volume measurement

Plasma volume was measured simultaneously with Evans' blue dye and transferrin bound Fe-59. The method of Gibson and Evelyn (14) was used for Evans'

blue plasma volume determination. Ten ml. of a 0.1 per cent solution of dye was injected intravenously. The concentration of dye in plasma samples was measured with a Beckman spectrophotometer, using a one-cm. light path, or with the micro attachment of the Evelyn colorimeter. Hemolyzed samples were discarded. The concentration of dye and the radioactivity present in the 10-, 20-, and 30-minute samples were plotted against time on semi-log paper and the curves extrapolated back to zero time. The concentration of dye and the radioactivity present at zero time were used for calculation of plasma volume. The values for plasma volume as measured by Evans' blue dye and transferrin bound Fe-59 agreed closely, the mean difference being 3 per cent. The lower of the two values was used for calculation of red cell mass.

Total blood volume and red cell volume were calculated from plasma volume and the mean of hematocrits of the blank, 10-, 20-, and 30-minute samples. Hematocrits were done by the method of Wintrobe (15) and spun at 2,000 G for 60 minutes. The observed hematocrit was corrected for trapped plasma (16) and by a factor of 0.91 to correct for the ratio of total body to venous hematocrit (17).

F. Erythrocyte survival studies

The technique of Ashby, as modified by Ebert and Emerson (18), was used. Type O blood was transfused into type A recipients or subtype N blood was transfused into subtype M recipients. The number of inagglutinable cells remaining in the recipient's circulation was determined at 7 to 14-day intervals until none remained. The recipient's cells were agglutinated by the use of potent, dried, powdered rabbit antisera (Lederle). The sample for counting was diluted 1:200 (0.1 ml. to 20 ml.) in sterile normal saline. A red cell count was performed on this suspension. Then approximately 0.2 ml. of this dilution was added to a small amount (10 to 20 mgm.) of the dry antiserum and shaken until all antiserum was dissolved. The tube was then spun at 500 r.p.m. for five minutes and the cells were resuspended by gentle flicking of the tube. This was repeated once and then the cell suspension was transferred to a counting chamber. A minimum of 500 unagglutinated cells was counted in each chamber when possible, and a minimum of 1,000 cells was counted for each sample. All counts were performed by the same technician, in duplicate. Black counts were performed on two separate occasions on all recipients, and only those subjects with inagglutinable cell counts of less than 20,000 per cubic mm. were used for this study. Blood was infused into recipients within 18 hours after collection into plastic bags (Fenwal) containing A.C.D. anticoagulant solution. The blood was given in the form of a replacement transfusion, a phlebotomy of 500 to 800 ml., being followed immediately by an infusion of 500 to 1,000 ml. of donor blood. Thereafter, the blank count was subtracted from the observed gross inagglutinable cell count to give the net inagglutinable cell count per cubic mm. of blood. The net inagglutinable cell count (y axis) was then plotted against time in days (x axis) on rectangular coordinate paper. A curve was then drawn through these points.

NoQ curves were constructed by the technique of DeGowin, Ellis, Sheets, Hamilton, and Janney (19), by the use of the following formula: $NoQ = n/(1 - [t/T])$. Where n = observed net inagglutinable cell count, t = time in days when the sample was drawn. However, rather than use an assumed value of 120 days for T as was done by the above authors, the observed intercept of the original curve with the time axis was used as T. NoQ values were calculated for every observed value of n and plotted on semi-log paper against time. These points described what appeared to be an exponential function. The rate of red cell destruction was calculated from these data. The percentage of red cells lost per day as a result of linear decay was computed from 1/T and the percentage of cells lost per day as a result of exponential decay was computed from ln 2/time in days required to remove 50 per cent of the donor cells by exponential decay alone (NoQ curve). The sum of these two values gave the total rate of red cell loss (per cent per day).

G. Fecal urobilinogen

This was measured by the technique of Schwartz, Sborov, and Watson (20), using an Evelyn photoelectric colorimeter calibrated with Pontacyl dye standards. All stools were collected for four-day periods and kept in opaque bottles under continuous refrigeration. Hemolytic indices were calculated by the method of Miller, Singer, and Dameshek (21).

H. Miscellaneous

Body surface area was determined from height and weight by the use of the DuBois nomogram (22). Hemoglobin was measured as oxyhemoglobin with an Evelyn photoelectric colorimeter which was calibrated on the basis of Van Slyke gas analysis. Sedimentation rates were determined by the Rourke-Ernstene method and expressed as the corrected blood sedimentation index (B.S.I.) in mm. per minute (23). Reticulocytes were stained with brilliant cresyl blue and counted by the dry method. Total circulating hemoglobin was determined by the product of the red cell mass and M.C.H.C. per 100. The total circulating hemoglobin iron was calculated from the total circulating hemoglobin using the value of 0.339 per cent as the iron content of hemoglobin (24).

RESULTS

A total of 42 patients and 10 control subjects was studied. The mean age of the patients was 53.3 years, and of the controls, 51.0 years. The subjects are listed by number in Table I.

A. Hematology (Table I)

The patients studied had a lower mean hemoglobin concentration (11.7 Gm. per cent) than the

controls (14.9 Gm. per cent). The female patients' mean hemoglobin (11.3) was lower than the male patients' mean hemoglobin (12.3). Likewise the female controls' mean hemoglobin (13.1) was lower than the male controls' mean hemoglobin (15.0). The values for hematocrit showed similar differences, the patients having a lower mean value (38.2 per cent) than the controls (43.1 per cent). The red cell counts also showed similar differences, the patients having lower mean values (4.50×10^6 per cubic mm.) than controls (4.76).

Computation of red cell indices revealed that the patients had a lower average Mean Corpuscular Hemoglobin (27.7 γγ) than the controls (29.5 γγ), a slightly lower average Mean Corpuscular Volume (89.9 cubic micra) than controls

TABLE I

No.	Age	WT. (Kg.)	SURF. AREA (Sq.M.)	HGB (Gms. %)	HCT %	RBC x 10^6/cm.	BLOOD SED. INDEX mm/min	RETICS %	PLASMA VOL. ml.		RBC Vol. ml.	TOTAL BLOOD VOL. ml.	PLASMA IRON TURNOVER RATE (mg./day)		RBC UPTAKE	
									EVANS BLUE	Fe^{59}			MOD. A	MOD. B	%	DAYS
							I. FEMALE PATIENTS									
1	62	49.7	1.49	11.80	38.5	4.34	1.0	1.6	—	2610	1309	3919	25.82	27.28	88.6	11
2	45	61.0	1.58	11.32	37.5	4.54	1.0	1.9	3153	3356	1484	4637	20.89	21.83	96.5	10
3	71	46.3	1.41	12.16	39.0	4.65	1.1	1.0	2112	2244	1054	3166	36.78	39.41	88.4	11
4	54	45.7	1.40	11.00	35.5	4.22	0.85	2.6	3184	3260	1358	4542	35.72	38.41	86.7	10
5	47	53.0	1.61	9.98	34.5	4.37	1.45	0.6	2803	2819	1230	4033	20.08	21.78	88.1	9
6	44	55.9	1.56	10.45	33.6	3.78	1.65	0.3	2741	2717	1267	3984	10.40	10.80	85.4	7
7	62	49.4	1.49	11.67	35.8	4.26	1.1	0.5	2587	2643	1179	3760	21.99	27.96	82.1	18
8	65	61.4	1.66	11.0	35.2	3.98	0.4	1.4	2964	2955	1266	4221	24.83	25.40	93.9	9
9	67	60.0	1.65	10.75	34.6	3.54	0.6	1.3	2047	2011	1131	3142	14.99	17.45	96.1	13
10	39	95.6	1.94	12.59	40.0	4.14	1.4	1.0	2970	3029	1476	4446	24.81	25.89	87.9	17
11	47	48.6	1.53	9.30	30.0	4.25	—	0.9	3061	3110	1009	4070	22.90	24.86	90.3	9
12	63	66.6	1.72	13.84	44.3	4.92	2.0	—	2829	2856	1387	4216	30.05	30.60	80.4	8
13	53	46.8	1.41	12.70	41.0	4.29	1.1	0.4	1944	1963	1161	3105	18.46	19.14	80.8	10
14	59	60.9	1.60	11.14	38.5	4.41	1.5	1.1	2301	2327	1093	3394	30.53	32.49	82.5	10
15	52	46.4	1.40	12.02	39.5	4.79	0.85	0.9	1988	2038	1015	3003	25.51	24.48	87.0	10
16	48	60.5	1.60	11.00	35.4	4.22	1.1	—	—	2463	1112	3575	15.04	16.38	88.9	12
17	54	39.6	1.33	10.90	36.2	4.29	1.8	0.7	—	2749	1128	3877	34.64	38.71	85.0	7
18	68	60.9	1.56	10.05	36.1	4.50	0.6	—	2437	2842	1250	3687	29.96	32.94	99.1	6
19	46	57.3	1.58	10.68	35.6	4.81	0.95	—	2458	2424	1104	3528	16.59	18.58	84.9	12
20	62	60.5	1.58	11.32	34.5	4.05	—	—	2306	2245	1018	3263	29.61	32.86	78.2	12
							II. MALE PATIENTS									
21	56	81.8	1.95	13.00	40.2	4.24	—	—	3541	3543	1763	5216	49.60	52.80	75.5	14
22	61	40.9	1.42	11.00	37.1	4.07	1.7	1.7	2295	2386	1263	3558	29.52	27.26	91.7	14
23	67	52.3	1.58	12.35	41.0	4.24	0.5	0.8	2913	3004	1534	4447	48.66	48.54	71.4	10
24	60	68.2	1.80	12.97	43.4	4.60	1.7	1.3	3557	3725	2027	5584	56.93	59.25	79.6	7
25	61	65.5	1.85	13.45	45.0	5.15	1.4	—	3176	3450	2022	5190	37.79	36.84	75.0	8
26	38	71.4	1.83	15.10	48.3	5.41	1.9	—	2505	2615	1385	3890	24.17	25.27	80.0	7
27	67	58.6	1.69	11.00	37.5	4.08	0.8	—	2531	2623	1480	4011	27.13	27.26	79.5	12
28	41	57.7	1.62	12.78	41.7	4.95	0.7	0.4	3015	2929	1570	4499	37.70	40.16	87.3	10
29	43	82.7	2.09	13.45	43.3	4.78	1.3	—	2934	2238	1784	4622	29.04	30.34	79.9	10
30	39	69.5	1.85	7.19	26.1	3.64	0.7	0.7	3668	4517	1059	4727	29.21	33.80	89.9	7
31	20	82.7	2.00	13.34	41.5	4.31	1.25	—	3554	3622	1823	5377	35.59	28.94	89.1	10
32	44	56.8	1.51	11.67	41.9	4.27	1.1	—	—	3700	1118	4818	29.88	30.12	89.2	7
							III. FEMALE CONTROLS									
1	30	50.9	1.50	13.05	39.5	4.17	0.2	—	2530	2436	1272	3808	23.79	29.36	79.0	9
2	63	61.8	1.65	14.02	43.4	4.72	—	—	2304	2085	1289	3374	22.30	29.16	72.5	17
3	43	68.2	1.66	13.34	41.6	4.54	—	—	2176	2291	1292	3470	26.76	34.95	90.6	17
4	64	75.0	1.79	11.67	36.0	4.26	—	—	2936	2950	1382	4318	23.56	27.68	84.8	6
5	56	74.1	1.86	13.45	41.8	4.41	—	—	2340	2557	1380	3720	17.95	21.26	75.6	16
							IV. MALE CONTROLS									
6	62	63.2	1.66	14.60	44.4	4.90	0.25	0.9	2554	2443	1575	4018	2412	25.43	83.6	13
7	35	69.5	1.85	16.50	50.5	5.68	0.2	—	2516	2583	1883	4399	23.41	27.33	78.4	16
8	34	90.0	2.06	15.00	46.5	5.47	0.1	2.0	3214	3227	2226	5440	33.00	36.24	75.0	7
9	65	60.5	1.63	15.00	46.6	4.82	—	—	2557	2529	1637	4166	33.74	37.62	77.3	14
10	58	84.1	1.99	13.71	40.8	4.61	0.45	—	3358	3637	1856	5214	34.15	37.25	86.2	14

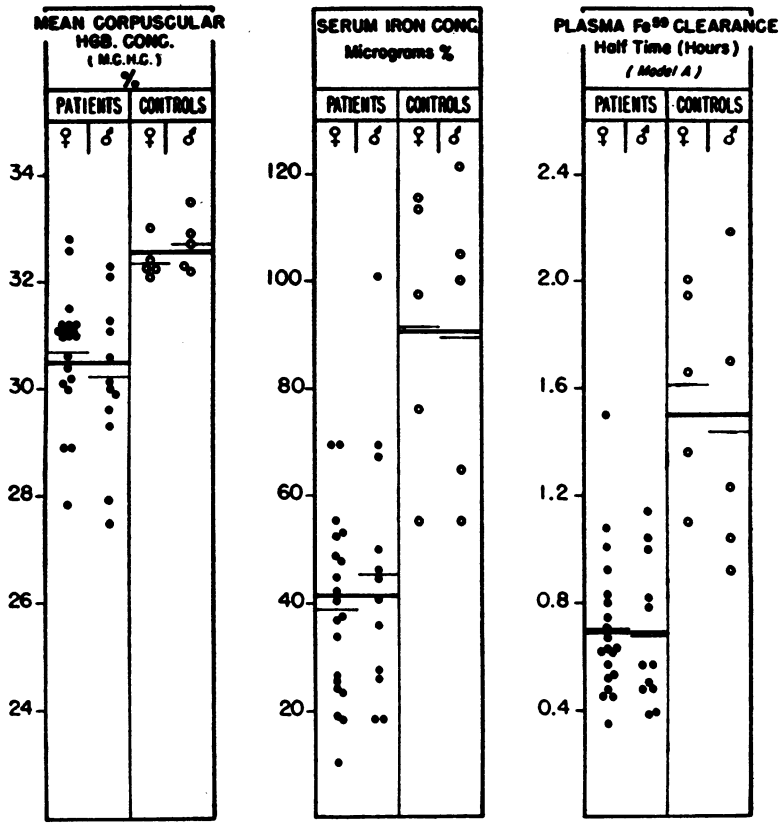

FIG. 2. VALUES OF M.C.H.C., SERUM IRON CONCENTRATION, AND HALF-TIME OF PLASMA FE-59 CLEARANCE IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

(90.8), and a lower average Mean Corpuscular Hemoglobin Concentration (30.5 per cent) than controls (32.6 per cent). It is of interest to note that 28 of the 32 patients studied had M.C.H.C. values below 32 per cent while none of the control subjects had a value below 32 per cent (Figure 2), indicating that the rheumatoid arthritis patients have a slightly hypochromic anemia. All patients showed an elevated erythrocyte sedimentation rate. No significant reticulocytosis was found in the patients. All of the patients studied had negative Coombs' tests.

B. Serum iron concentration

The mean serum iron concentration in the patients was significantly lower than that of the controls, the mean value for patients being 41.5 micrograms per cent and for the controls 90.5 micrograms per cent. Whereas a wide range of values was found in both groups (Figure 2), the standard error of the difference between the means was 8.80, giving a t value of 5.56. The probability that so large a difference between means was due to chance alone (p) is less than 0.001.

C. Radio-iron studies

1. Plasma radioiron removal rate. The period of time in hours required for the removal from the plasma of one-half of the transferrin-bound radioiron is referred to as the "half-time of plasma radioiron removal" (clearance). Using Model A, the mean half-time for the patients (0.70 hour) was definitely shorter than for the controls (1.53 hours). The distribution of these values is shown in Figure 2. The difference between these means is highly significant, the standard error of the difference being 0.137, the t value 6.05, and the p value less than 0.001. A definite linear relationship was found to exist between the serum iron concentration and the half-time of plasma radio-

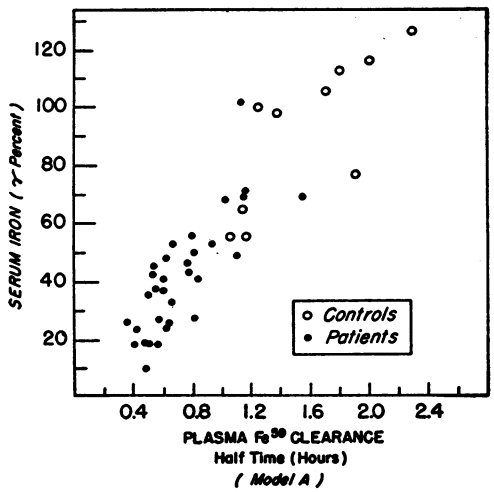

FIG. 3. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND HALF TIME OF PLASMA FE-59 CLEARANCE
$r = 0.77$, $p = < 0.001$.

iron clearance (Figure 3), the coefficient of correlation (r) being 0.775, $p < 0.001$. This indicates that in the subjects studied a lower serum iron concentration is associated with a more rapid rate of removal of iron from the plasma.

Using Model B gives a slightly more rapid rate of plasma radioiron removal. The mean value for half-time of plasma iron removal for patients was 0.65 hour and for controls 1.27 hours. The difference between these means was also significant, p being less than 0.001.

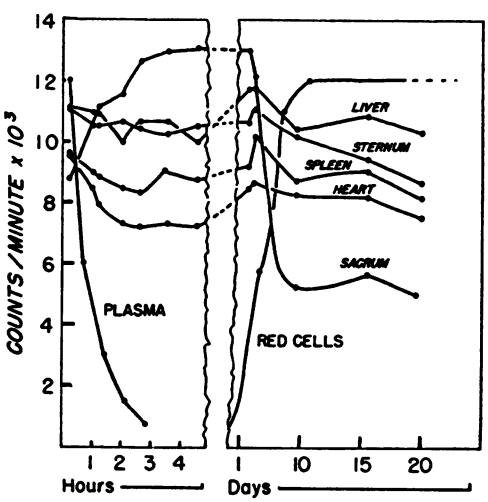

FIG. 4. EXTERNAL BODY MONITORING IN A RHEUMATOID ARTHRITIS PATIENT

Values for plasma and red cells were determined from measurement of blood samples. All other values were determined by external probe counting over indicated sites.

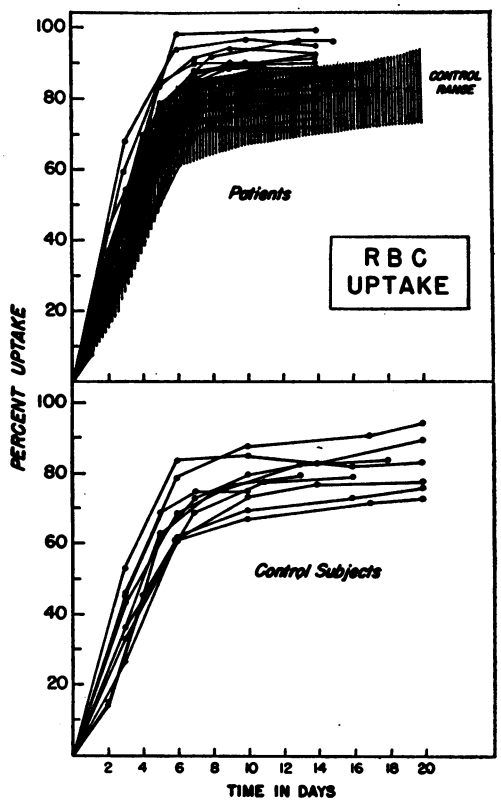

FIG. 5. RED CELL INCORPORATION OF INTRAVENOUSLY INJECTED RADIOIRON

2. External body monitoring. These studies were performed on 11 patients, 7 females and 4 males. All patients showed qualitatively the same

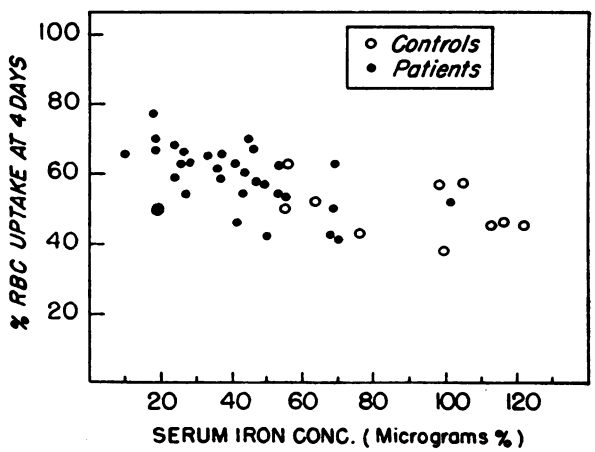

FIG. 6. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND THE RATE OF RED CELL UPTAKE OF RADIOIRON

The per cent uptake at four days is used as an index of the rate of incorporation of radioiron into red cells. $r = -0.63$, $p = < 0.001$.

Table II

	Model A			Model B		
	MEAN VALUE		P	MEAN VALUE		P
	Patients	Controls	Difference Pts. vs. Controls	Patients	Controls	Difference Pts. vs. Controls
PLASMA IRON TURNOVER RATE						
Mg. / Day	28.90	26.28	<.40	30.24	30.63	<.90
Mg. / Kg. / Day	.496	.383	<.02	.524	.448	<.20
Mg. / Sq.M. / Day	17.77	14.94	<.10	18.63	17.47	<.50
Mg. / 100 mL TBV / Day	.701	.631	<.20	.736	.741	>.90
Mg. / 100 mL RBC / Day	2.17	1.69	<.01	2.28	1.98	<.20
RBC IRON TURNOVER RATE						
Mg. / Day	24.44	21.15	<.20	25.59	24.67	<.70
Mg. / Kg. / Day	.417	.307	<.001	.445	.360	<.05
Mg. / 100 mL RBC / Day	1.85	1.36	<.001	1.95	1.60	<.05
Mg. / 100 mg. Hgb Fe / Day	1.79	1.21	<.001	1.89	1.42	<.01

pattern (Figure 4). The major change in activity occurred at the marrow site (sacrum), a rapid rise occurring during the first 4 to 5 hours after injection of the radioiron, followed by a slow continued rise during the next 24 hours. Thereafter, a rapid decline in activity occurred over the ensuing 3 to 6 days. The counting activity over the liver, spleen, and sternum reflected, in general, the changes observed in the blood measured over the heart, where a moderate fall in activity was observed during the first 24 hours followed by a moderate rise during the succeeding 3 to 7 days. This indicated that the major portion of radioiron removed from the plasma was deposited in the bone marrow, whence it was discharged into the blood in newly formed red cells. No evidence was found in these patients to suggest a diversion of plasma radioiron to other organs such as liver and spleen. The ferrokinetic pattern found in these patients was comparable to that found in normal subjects (25).

3. Red cell uptake of radioiron. The rate of radioiron incorporation into red cells is shown in Figure 5. Both patients and controls showed rapid uptake by red cells of 70 to 95 per cent of the injected radioiron in the 7 to 10 days following injection. The curves for the patients show a slightly more rapid initial slope of uptake, suggesting some relationship between serum iron concentration and the rate of red cell uptake. This relationship is shown in Figure 6, the percentage red cell uptake at 4 days serving as a measure of uptake rate. Subjects with lower serum iron concentrations showed more rapid uptake of injected radioiron. A similar relationship existed between the per cent uptake at 3, 5, or 6 days and the serum iron concentration.

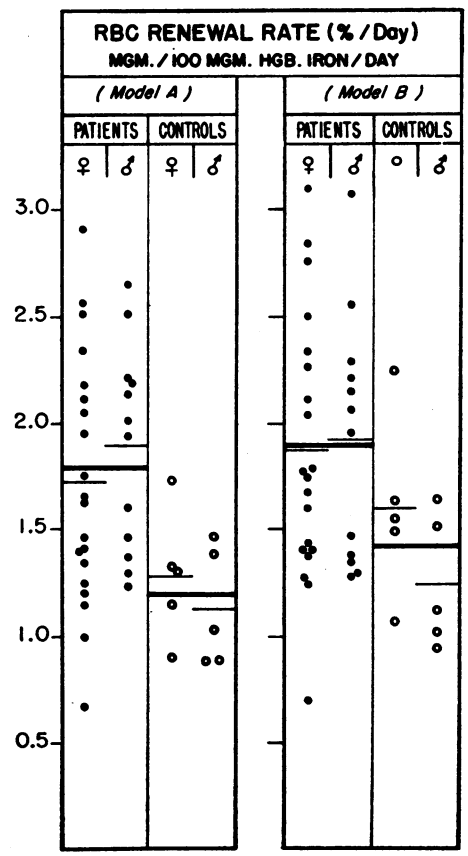

FIG. 7. VALUES FOR RED CELL RENEWAL RATE IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

4. Iron turnover rates (*Table II*). Using Model A, the mean value for the mgm. of plasma iron turned over per day was slightly but not significantly higher for patients than for controls. In both patients and controls, the mean values for males were definitely higher than for females, reflecting the larger body size of the males. When the iron turnover rates were corrected for body size the values for males and females were comparable. The mean plasma iron turnover rate corrected for body weight was significantly higher for patients than for controls. However, when the turnover rates were corrected for other parameters of body size such as surface area and total blood volume, the mean values for patients were not significantly higher than those for controls. There was no relationship between the serum iron concentration and the computed values for plasma iron turnover rate, the coefficient of correlation being 0.06, with a standard error of 0.15. Similarly, no correlation existed between serum iron concentration and plasma iron turnover rate corrected for body weight, nor between the half-time of plasma radioiron removal rate and plasma iron turnover rate.

The values for red cell iron turnover rates showed differences between patients and controls which were similar to those for plasma iron turnover rates. The rate of renewal of the circulating red cell mass can be computed by correcting the red cell iron turnover rate for red cell mass since one ml. of packed red cells contains approximately one mgm. of iron. The mean red cell renewal rate (mgm. per 100 ml. red cell mass per day) was significantly higher for patients than for controls. Because the patients had a lower average Mean Corpuscular Hemoglobin Concentration than controls, a more exact comparison of red cell iron renewal rates would be obtained on the basis of circulating hemoglobin iron. When red cell renewal rates were expressed as mgm. per 100 mgm. hemoglobin iron per day, the difference between patients

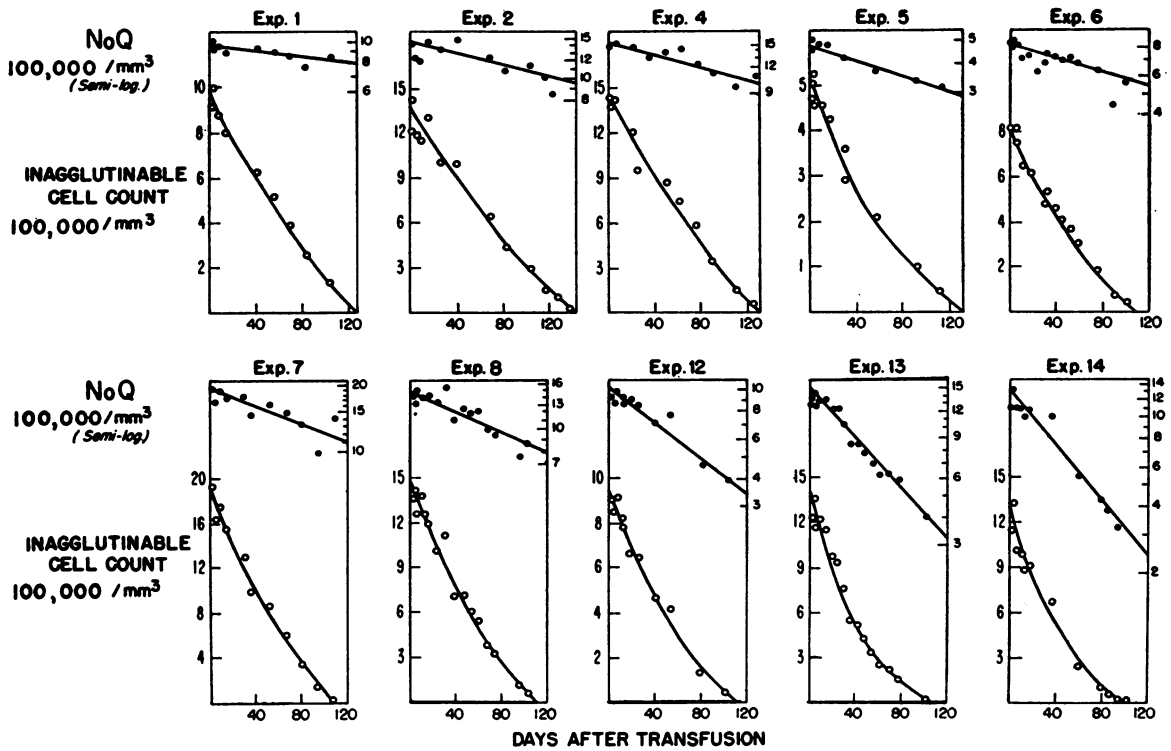

FIG. 8. SURVIVAL OF NORMAL RED CELLS IN RHEUMATOID ARTHRITIS PATIENTS

In each experiment the observed net inagglutinable cell counts (open circles) are plotted on Cartesian Coordinates in the lower curve, while the computed NoQ values (solid circles) are plotted on semi-log coordinates in the upper curve. Experiments are numbered in order of increasing abnormality. Experiments Nos. 3, 9, 10, and 11 are omitted and shown in Figure 9.

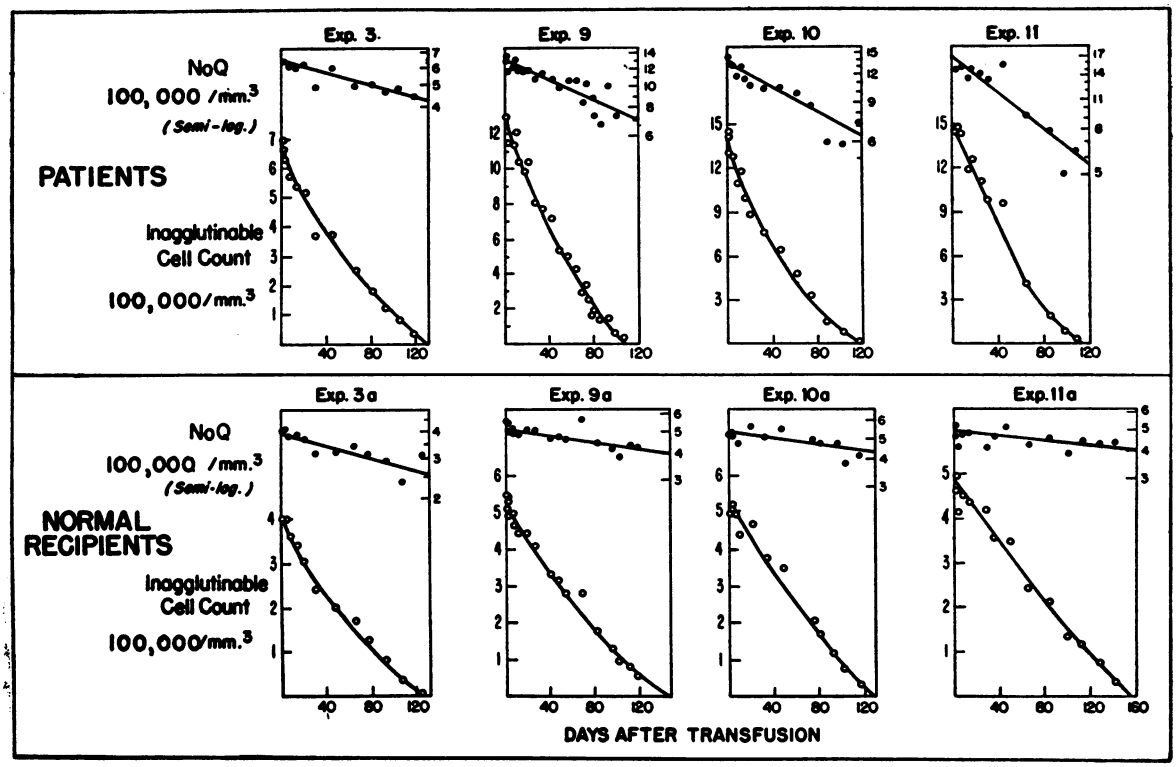

FIG. 9. SURVIVAL OF RHEUMATOID ARTHRITIS PATIENTS' RED CELLS IN NORMAL RECIPIENTS

The data on survival of patients' red cells in normal recipients are placed immediately below the data on survival of normal red cells in their respective donor patients. Thus, the patient of Experiment 11 served as the donor for the red cells whose survival in a normal recipient is shown in Experiment 11a.

and controls was greater, the mean value being significantly higher in patients.

Using Model B for computation of iron turnover rates gives slightly higher values than were obtained using Model A. This effect was more marked in controls than in arthritis patients, resulting in a decrease in the differences between means. The mean values obtained for plasma iron turnover rate using Model B show no significant differences between patients and controls, with or without corrections for body weight, surface area, blood volume, or red cell mass, p values exceeding 0.1 in all instances. However, the rate of red cell renewal or the red cell iron turnover rate expressed either as mgm. per 100 ml. of red cell mass or mgm. per 100 mgm. hemoglobin iron is still significantly greater for patients than for controls (Figure 7).

D. Erythrocyte survival studies

The survival of red cells from normal donors was studied in 13 rheumatoid arthritis patients (Figures 8 and 9). Eight of the patients were females and five were males. One male patient was studied on two separate occasions. These experiments are numbered in order of increasing abnormality of cell survival. In Experiment No. 1, the inagglutinable cell counts declined at an even rate to the baseline in about 120 days, indicating predominantly linear ("senescent") loss of red cells, and the NoQ curve is virtually horizontal, indicating minimal exponential ("random") red cell destruction. In successive experiments the inagglutinable cell count curves are increasingly curvilinear and the NoQ curves progressively more vertical, indicating greater degrees of random red cell destruction.

In four cases we were able to study simultaneously the survival of the patients' red cells in a normal recipient (Figure 9). None of the normal recipients developed any symptoms or signs of rheumatoid arthritis following transfusion of blood from the patients. One recipient developed a transient febrile episode, but otherwise the trans-

Table III

RATE OF RED CELL DESTRUCTION

SURV. EXP. NO.	PATIENT NO. (Table I)	LINEAR LOSS T	LINEAR LOSS %/D	EXP. LOSS Half Time (Days)	EXP. LOSS %/D	TOTAL LOSS %/D
		PATIENTS				
1	9	125	0.80	507	0.14	0.94
2	23	140	0.71	240	0.29	1.00
3	31	127	0.79	230	0.30	1.09
4	12	132	0.76	178	0.39	1.15
5	25	130	0.77	177	0.39	1.16
6	26	107	0.93	178	0.39	1.32
7	13	109	0.92	163	0.43	1.35
8	6	112	0.89	139	0.50	1.39
9	22	107	0.93	152	0.53	1.46
10	26	118	0.85	112	0.62	1.46
11	17	112	0.89	78	0.89	1.77
12	11	114	0.88	77	0.90	1.77
13	1	108	0.93	53	1.31	2.23
14	5	104	0.96	48	1.44	2.39
	DONOR PATIENT	RECIPIENTS				
3A	3	127	0.79	220	0.31	1.10
9A	9	137	0.73	410	0.17	0.90
10A	10	127	0.79	410	0.17	0.96
11A	11	153	0.65	537	0.13	0.78

fusions were uneventful. These experiments are indicated by the letter "a" and numbered to correspond with their respective donor patient. Thus, the patient studied in Experiment No. 3 served as the red cell donor for Experiment No. 3a. In all four normal recipients the inagglutinable cell count curves were virtually linear and the NoQ curves almost horizontal, indicating predominantly linear ("senescent") loss of the transfused patients red cells. In contrast, three of the donor patients showed an increased "random" loss of normal red cells (Experiments Nos. 9–11).

The rates of red cell destruction computed from these data are listed in Table III. The mean rate of red cell loss in the patients studied was 1.46 per cent per day, ranging from 0.94 per cent per day, a value within the control range, to 2.39 per cent per day, more than double the control rate. This increased rate of red cell loss was predominantly the result of an increased rate of exponential loss of red cells. The rates of linear loss showed only small differences. The mean rate of red cell loss in the normal recipients was 0.97 per cent per day, ranging from 0.78 per cent to 1.10 per cent per day. All the normal recipients showed predominantly linear loss of red cells. The red cells lost at the lowest rate in the normal recipient (Experiment 11a) came from the donor patient in whom the rate of loss of normal cells was highest of the four donor patients, while the cells lost at the highest rate in the normal recipient (Experiment No. 3a) came from the patient in whom the rate of loss of normal cells was lowest of the donor patients. The patient studied on two separate occasions showed a slightly lower rate of cell loss in the second study (Experiment No. 6) performed 5 months after the first study (Experiment No. 10).

E. Fecal urobilinogen excretion (Figure 10)

Forty-six measurements of fecal urobilinogen excretion were carried out in 21 patients. Fifty-five per cent of these values were below 50 mgm. per day, and only three values were above 150 mgm. per day, all obtained on the same patient. Many of the patients studied were at complete bed rest, and as a result chronically constipated despite the use of saline cathartics. Accordingly, many four-day stool specimens weighed less than 300 Gm. and yielded low values for fecal urobilinogen. In addition, any errors of measurement (*e.g.*, incomplete reduction or extraction in benzene) resulted in lower recoveries of urobilinogen. To

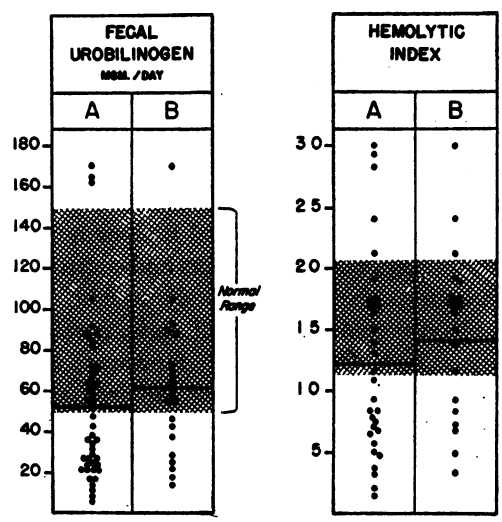

FIG. 10. FECAL UROBILINOGEN EXCRETION IN RHEUMATOID ARTHRITIS PATIENTS

All of the values obtained are shown in Column A. The highest value of repeat determinations in any given patient is shown in Column B.

minimize these effects only the highest value obtained on repeat samples in a given patient are shown in scattergram B, Figure 10. Using this method, 38 per cent of the values were below 50 mgm. per day and only one above 150 mgm. per day. The mean value is still in the normal range.

Rates of urobilinogen excretion were corrected for red cell mass and expressed as hemolytic indices. The mean of all the determinations (12.1) was in the low normal range. Using only the highest value obtained on each patient, the mean value (14.2) fell within the normal range.

DISCUSSION

A. Iron metabolism

This study indicates that rheumatoid arthritis patients utilize circulating transferrin-bound plasma iron for erythrocyte production in a normal manner. Seventy to ninety-five per cent of the plasma iron is utilized for red cell production and no significant amount of iron is diverted from the plasma to organ sites other than the bone marrow. In the patients studied, the plasma radioiron was removed more rapidly from the plasma and incorporated more rapidly into red cells than it was in the normal subjects. This difference may be explained on the basis that the radioiron was introduced into a smaller plasma iron pool in the patients, since the lower the serum iron concentration, the more rapid the rate of removal from the plasma and the rate of incorporation into red cells of the injected radioiron.

Previous studies of iron metabolism in patients with chronic inflammation, including patients with rheumatoid arthritis, have shown diminished red cell utilization (26–28), and increased tissue deposition of injected radioiron (29). These studies differed from the present one in that the injected radioiron was not bound to transferrin, and the amounts of carrier iron injected often exceeded the total iron-binding capacity of the plasma. More recent studies using trace quantities of radioiron in patients with chronic infection (30) and rheumatoid arthritis (31) have given results similar to ours. Thus, while patients with chronic inflammation show unimpaired red cell uptake of circulating plasma iron, they do show impaired uptake of relatively large quantities of unbound iron, the greatest proportion of which is deposited in the reticulo-endothelial tissues. This suggests that the release of iron from the reticulo-endothelial tissues to the plasma may be impaired in these patients. Confirmation of this hypothesis has recently been obtained in animal experiments which indicate that in the presence of sterile inflammation the rate of reutilization of iron from non-viable red cells is reduced, whereas the utilization of transferrin-bound plasma iron is not impaired (32). A diminished rate of release of iron from the reticulo-endothelial system to the plasma, coupled with a normal rate of migration of iron from the plasma to erythropoietic tissue, would adequately explain the low plasma iron concentration that is so consistently found in association with rheumatoid arthritis and other inflammatory states.

The total amount of iron turned over per day through the plasma and into the red cells was not significantly different for the patients and the controls. When the turnover rates were compared on the basis of body weight, the rates for patients were significantly greater than for controls. However, because the patients are chronically ill and are poorly nourished, body weight could be criticized as an unsatisfactory basis for comparison with normals. This was emphasized by the fact that the use of body surface area or total blood volume as a basis for comparison showed no significant difference between patients and controls. Thus, the mean rate of hemoglobin production was similar for patients and controls. Since the patients had a decreased circulating red cell mass and a normal rate of hemoglobin production, it follows that the rate of red cell renewal should have been increased. Computation of red cell renewal rates did give significantly higher rates for patients than for controls, the patients showing a mean renewal rate 1.5 times that of controls. Since the patients had fairly constant hemoglobin levels, the rates of red cell destruction were probably similar to the rates of cell renewal. This indicates that the patients have a higher rate of red cell destruction, or expressed in another way, a shorter red cell life span than the controls.

As described above, the turnover rates for plasma and red cell iron have been computed using the model proposed by Huff and his co-workers (10) (Model A). This model describes plasma radioiron clearance as an exponential function of time. However, careful inspection of our

data revealed a slight but definite curvilinearity, as has also been reported by Sharney, Schwartz, Wasserman, Port, and Leavitt (33). The mean curvature was found to be greater for patients ($c = 5.38 \times 10^{-5}$) than for controls ($c = 4.56 \times 10^{-5}$). For this reason Model B was developed and introduced. This model probably does not have great biological significance but it does allow an estimate to be made of the effect of this curvature on the observed differences. Accordingly, the turnover rates were recalculated using Model B in which plasma radioiron removal is assumed to be curvilinear. While the resulting differences between patients and controls were slightly decreased, these data confirmed the findings derived from Model A. Again, plasma iron turnover rates were not significantly different for patients and controls, while the red cell renewal rates were significantly greater in patients than controls.

These estimates of red cell production rates are based on data obtained during the morning hours only. A diurnal variation in plasma iron concentration (34, 35) and plasma iron turnover rate (36) has been demonstrated. It is possible that this diurnal variation is sufficiently different for patients and controls to alter the data presented. However, the rate of plasma iron removal was related to the plasma iron concentration, suggesting that a decrease in plasma iron concentration would be associated with an increased rate of removal of iron from the plasma. The rate of plasma iron turnover was not related to the plasma iron concentration suggesting that a decrease in plasma iron concentration would not be associated with any consistent change in plasma iron turnover rate. In addition to diurnal variation, errors in measurement of blood volume, serum iron levels, plasma radioiron clearance rates and perhaps unknown factors, may influence the computation of red cell renewal rates. As a result, the use of this method for detecting moderate alterations of red cell production in individual instances is often difficult. However, in the present study, patients and controls were studied under identical conditions and subject to similar errors. The number of subjects studied, the high level of significance of the difference between patient and control groups, and the confirmation of these data obtained with the use of Model B would indicate that the patients studied do show a moderate increase in red cell renewal rate when compared to controls.

B. Red cell survival

More than half of the rheumatoid arthritis patients studied showed increased rates of destruction of normal red cells. This increase in erythrocyte destruction was primarily the result of increased random loss of red cells. The potential life span of the red cells was not significantly shortened. When the cells from an arthritis patient were transfused into a normal recipient they showed a normal survival. This would indicate that the patients' red cells are not intrinsically defective, and that a defect extinsic to the red cell is responsible for the shortened survival in the patient.

If the patient's own red cells are destroyed in the same manner as the transfused normal cells, then patients showing increased rates of random red cell destruction should have a cell population containing a higher proportion of young red cells. When the younger red cell population is transfused into a normal recipient the rate of red cell loss should be less than normal. The data obtained from transfusion of patients' cells into normal recipients suggest that this was actually the case. The destruction rate of patients' cells was lower in the normal recipients when the destruction rate of normal cells was faster in the donor patient. A recent study by Alexander, Richmond, Roy, and Duthie (7) has also shown shortened survival of normal red cells in arthritis patients. They found in addition that red cells from an arthritis patient when transfused into another arthritis patient showed a cell survival which was better than that of normal red cells in that patient. These findings are consistent with the hypothesis that the arthritis patients have a cell population containing a greater proportion of young cells which show better survival than blood from a normal donor containing a normal cell population of all ages. These observations suggest that rheumatoid arthritis patients frequently show increased rates of destruction of their own circulating red cells. Similar findings of shortened red cell survival have been reported in anemias complicating cirrhosis, uremia (37, 38), and malignant diseases (39, 40). No immune mechanism could be

detected, and the cause of the shortened cell survival is not known.

Normal cells have been found to disappear in approximately linear fashion at a rate of 0.85 to 1.0 per cent per day in normal subjects. Some females who are apparently normal show slightly shortened red cell survival (41). This could account in part for the greater rates of red cell destruction found in the female patients in this series. However, half of our male patients showed increased destruction, while three-quarters of the females showed abnormal survival. Cells from a female patient were capable of normal survival when transfused into a normal recipient. Finally, a decrease in red cell survival regardless of cause would require an increase in red cell production to maintain normal hemoglobin levels.

When destruction rates estimated from survival data were compared with renewal rates estimated from radioiron data in each individual patient studied, a poor correlation was found, the coefficient of correlation being 0.3. Because of the small number of patients studied with both techniques, the 95 per cent confidence limits of this coefficient ranged from -0.2 to 0.7. The inadequacy of the sample size for such a correlation is emphasized by the fact that elimination of a single subject (Survival Exp. No. 2) results in a correlation coefficient of 0.5. Thus, these data do not establish the presence or absence of a positive correlation between these measurements. In addition, the study was not designed to test this relationship, since these two measurements were made at different times, separated by a 2 to 3-week interval. Moreover, these measurements were made over very different periods of time, the radioiron measurements being made over a 4 to 6-hour period, while the survival measurements were made over a 4-month period. Only if the rates of cell production and destruction were constant for the 5-month period of study, or if these two measurements were made simultaneously over similar periods of time, could such a correlation be expected. For these reasons, data obtained with each method in patients were compared with controls studied with the same method, under identical conditions. When this was done the data obtained from the survival studies are in agreement with the radioiron data, indicating a moderate increase in rates of cell destruction in the patients studied. The mean rate of cell destruction computed from survival studies was 1.55 times greater for patients than for controls and the mean rate of red cell renewal computed from radioiron data was 1.48 times greater for patients than for controls.

C. Fecal urobilinogen excretion

The rheumatoid arthritis patients were found to excrete normal to decreased quantities of fecal urobilinogen. Similar findings have been previously reported (8, 42). This indicates that normal amounts of hemoglobin are destroyed per day in the patients, which confirms the findings of the radioiron studies. It should be emphasized that the increased rates of cell renewal and destruction found with the radioiron and survival techniques do not necessarily indicate that an increased quantity of red cells are destroyed, since an increased percentage of cells destroyed together with a smaller red cell mass could result in a normal or even decreased quantity of hemoglobin breakdown. When urobilinogen excretion was related to red cell mass and expressed as hemolytic index, the mean value for the patient group is within the normal range. This does not agree with the above findings of increased rates of red cell renewal and destruction. However, chronic constipation in the bed-ridden arthritis patients could have resulted in falsely low values for fecal urobilinogen (43). Moreover, this method may not be sufficiently sensitive or accurate to detect the moderate increase in rates of hemoglobin destruction demonstrated by the other methods.

D. The mechanism of the anemia in arthritis patients

The mean values obtained in our patients indicate that the rate of cell destruction was increased while the quantity of red cell production was comparable to normal controls. Anemia results because erythropoiesis fails to increase in order to compensate for the increased hemolysis. While this describes the average arthritis patient, there were patients with normal red cell life span and diminished cell production. There were other patients showing shortened red cell life span associated with an increase in cell production, which, however, was inadequate to compensate for the increased rate of destruction. In all of these situ-

ations the bone marrow fails to respond adequately to the stimulus of anemia. Increased hemolysis is an additional factor in the production of anemia, however, since red cell production in normal marrow can increase 6 to 8 times (44), it is apparent that red cell production does not increase in the arthritic patient to a degree possible in the normal.

A mild but definite hypochromia of the red cells was present in the arthritis patients in the present study, as well as in other studies (1, 42, 45). Thus, the anemia of arthritis resembles morphologically the anemia of iron deficiency. Other similarities of these two anemias include a consistently decreased serum iron concentration, rapid removal of iron from the plasma, increase in serum copper, increase in free erythrocyte protoporphyrin, low serum bilirubin, and low fecal urobilinogen excretion (2). These similarities suggest that a diminished supply of iron for erythropoiesis may play a role in the impaired marrow function in these patients, despite adequate tissue iron stores (46). Failure of iron to be released from the reticulo-endothelial cells to the plasma at a normal rate could be responsible for hypoferremia and a decrease in the amount of iron available for red cell production. That this abnormality in iron metabolism does contribute to the anemia is indicated by the fact that a significant correlation was found to exist between the degree of hypoferremia and the severity of the anemia in our patients (Figure 11). This has also been found in other studies (1, 2). Moreover, if a diminished supply of iron to the plasma plays a role in the production of anemia, then correction of the hypoferremia for prolonged periods of time should improve the anemia. While studies of the effect of iron therapy have been variable, several investigators using large doses of intravenous iron given over prolonged periods have reported definite improvement in the anemia (42, 45, 47). Since the characteristics of the anemia of arthritis are similar to those of the anemia associated with chronic infection and inflammation, and to other secondary anemias (2), similar mechanisms may exist in these conditions.

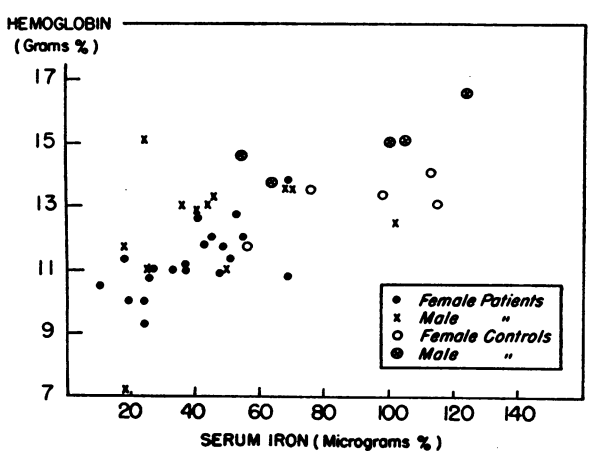

FIG. 11. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND HEMOGLOBIN LEVEL

$r = 0.68$, $p = < 0.001$.

CONCLUSIONS

Patients with rheumatoid arthritis show normal, unimpaired utilization of plasma iron for erythrocyte production. The amount of erythrocyte hemoglobin produced by these patients is approximately the same as in normal subjects. However, rates of red cell destruction and red cell renewal are abnormally increased. Increased red cell destruction results primarily from a greater random destruction of cells, the potential life span of the cells being virtually normal. The patients' erythrocytes proved capable of normal survival in a normal individual, indicating that the hemolytic mechanism in this disease is based on some factor associated with the red cell environment rather than on a defective quality of the red cell. Anemia reflects the failure of their bone marrow to respond adequately to the increased need for erythrocyte production. A defect in the release of iron from the R.-E. tissues to the plasma, resulting in hypoferremia and an inadequate supply of iron for red cell production, contributes to the impaired capacity of the bone marrow for red cell production.

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