PLASMA IRON TURNOVER IN NORMAL SUBJECTS

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Ferrokinetic measurements have been used extensively to characterize erythropoiesis (*1*). More specifically, the plasma iron turnover has been used to indicate the number of red-cell precursors over total erythropoiesis. This measurement, carried out at one interval of time, is extrapolated to the 24-hr period. Past studies have shown variations in repeated determinations of plasma iron turnover but no consistent variations during the day (*2–4*). The purpose of this study was to determine the amounts of variation in plasma iron turnover over the 24-hr period and to clarify insofar as possible the reasons for such variations.

MATERIALS AND METHODS

All subjects were healthy adult males between the ages of 21 and 60 without anemia or a history of blood donation in the preceding 6 months. They were divided into three groups: 19 subjects in Group 1 studied at 7 am and 7 pm, 18 subjects in Group 2 studied at 1 pm and 1 am and eight subjects in Group 3 studied at 6-hr intervals over the 24-hr period. These three studies were performed at widely varying times: the first in September of 1966, the second in January of 1967 and the third in January of 1968. During all studies normal activity was maintained as much as possible. With the aid of indwelling scalp vein needles, individuals in Group 3 were studied while asleep. In the latter group a total of 160 ml of blood was removed during this study.

The half-time of radioiron disappearance was determined after the intravenous injection of 5 μCi of radioiron as a citrate salt. Subjects of Group 3 received increasing amounts to a total of 0.25 μCi/kg. Plasma samples were obtained just prior to the injection of isotope for determination of plasma iron and radioactivity and 5, 30, 60, 90 and 120 min thereafter in Groups 1 and 2. In Group 3, 0- and 120-min samples were drawn in triplicate. Plasma iron was determined by the method of Bothwell and Mallet (*5*). Counting was performed in a standard gamma-detection system on a 2-ml aliquot of plasma with sufficient counts to provide a counting error of less than 3%.

Plasma iron turnover was calculated from the slope of radioiron disappearance and plasma iron and expressed as milligrams of iron per 100 ml whole blood per day (*6*). The plasma iron level for Groups 1 and 2 was obtained from the 0 value of a line determined by least-squares analysis of the observed plasma iron values. In Group 3 the average of the triplicate 0 value was used. In this group a 24-hr plasma iron curve was also constructed from the 0- and 120-min plasma iron values obtained eight times during the 24 hours.

Numerical differentiation of the plasma iron-level curve then provided a study of the dynamics of plasma iron. The derivative represents total flow of iron made up of the flow-in minus the flow-out. But the outflow can be measured from the disappearance of tracer, and thus one can get the whole picture of iron inflow, outflow and balance as a function of time.

RESULTS

A total of 106 observations on plasma iron, disappearance rate and plasma iron turnover was carried out on 45 subjects. The results are given in Tables 1, 2 and 3. Changes in plasma iron concentration measured over a 2-hr period of study on subjects of Groups 1 and 2 are shown in Fig. 1. The mean change in plasma iron was 3.7 μg/100 ml plasma/hr in Group 1 and 5.02 in Group 2. Median change in plasma iron in both groups was 3 μg/100 ml plasma/hr. Changes of more than 6 μg occurred 14 times and over 10 μg occurred five times in the 72 studies performed. The direction of change at 7 am was -1.23 μg/hr and at 7 pm -3.30 μg/hr; at 1 pm it was -3.62 and at 1 am -1.53.

The mean initial plasma iron turnover in subjects of Group 1 was 0.62 mg/100 ml whole blood/day, in Group 2, 0.80 and in Group 3, 0.87. The mean of all 45 initial plasma iron-turnover measurements was 0.76 ± 0.17. The mean of all plasma iron-turn-

Received May 1, 1969; original accepted Oct. 28, 1969.

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over measurements performed was 0.75 ± 0.19. Individual values ranged from 0.34 to 1.26 mg/100 ml whole blood/day.

Repetitive determinations at 12-hr intervals were performed in subjects of Groups 1 and 2. In Group 1 the mean plasma iron turnover at 7 am was 0.61 and at 7 pm was 0.54 mg/100 ml whole blood/day. In Group 2 the mean value at 1 pm was 0.80 and at 1 am 0.76 mg/100 ml whole blood/day. When the paired values of each subject were examined, a mean change regardless of direction in Group 1 of 0.10 was found and in Group 2 of 0.19. The mean difference, taking into account the direction of the change was +0.07 for Group 1 and −0.04 for Group 2. These differences analyzed as a "t" value (7) were significant to less than 0.005 and 0.001, respectively.

The relationship between the plasma iron level and turnover in the entire group of subjects was examined (Fig. 2). There appears to be a tendency for plasma iron turnover to be higher at higher levels of plasma iron. The relationship could be examined further in the repeated studies on individual subjects. The plasma iron turnover changed in the direction of plasma iron in 17 of 19 studies in Group 1, in 15 of 18 studies in Group 2 and in 24 of 32 studies in Group 3. Least-squares analysis of these data indicated a change of 0.036 mg/100 ml whole blood/day in plasma iron turnover for a variation in plasma iron of 10 μg/100 ml. This relationship is further examined in Fig. 3 by plotting changes in plasma iron from the initial level against change in plasma iron turnover from the initial value.

TABLE 1. PLASMA IRON TURNOVER IN NORMAL SUBJECTS STUDIED AT 7 AM AND 7 PM

	0 PI		$T_{1/2}$		PIT	
Subject	7 am	7 pm	7 am	7 pm	7 am	7 pm
1	64	83	81	87	0.470	0.564
2	98	66	105	81	0.564	0.485
3	173	52	125	57	0.943	0.530
4	119	92	84	86	0.863	0.666
5	55	51	47	55	0.675	0.548
6	122	64	115	68	0.597	0.543
7	116	62	109	69	0.614	0.531
8	55	76	55	70	0.558	0.616
9	89	63	85	73	0.633	0.534
10	113	70	84	62	0.739	0.698
11	102	51	90	61	0.638	0.509
12	93	78	86	77	0.624	0.608
13	111	60	119	71	0.602	0.554
14	98	44	94	55	0.601	0.476
15	56	56	101	88	0.338	0.391
16	79	50	80	53	0.592	0.557
17	73	83	96	104	0.474	0.530
18	117	75	105	81	0.643	0.538
19	63	42	86	66	0.443	0.393
Mean	95	64	92	72	0.611	0.541

TABLE 2. PLASMA IRON TURNOVER IN NORMAL SUBJECTS STUDIED AT 1 PM AND 1 AM

	0 PI		$T_{1/2}$		PIT	
Subject	1 pm	1 am	1 pm	1 am	1 pm	1 am
1	92	76	77	63	0.803	0.679
2	65	68	44	67	0.917	0.600
3	85	94	50	49	0.991	1.163
4	72	152	65	69	0.649	1.256
5	78	63	50	54	1.000	0.711
6	90	78	71	100	0.778	0.482
7	82	77	54	57	0.820	0.677
8	82	67	60	50	0.836	0.804
9	51	74	50	60	0.620	0.703
10	48	55	37	53	0.771	0.604
11	111	95	87	87	0.828	0.657
12	65	61	72	54	0.574	0.700
13	71	70	50	51	0.897	0.857
14	67	68	47	46	0.831	0.872
15	77	54	44	44	0.945	0.704
16	74	59	58	58	0.813	0.588
17	46	58	39	40	0.689	0.823
18	69	90	60	60	0.679	0.876
Mean	72	76	56	59	0.802	0.764

TABLE 3. PLASMA IRON TURNOVER IN NORMAL SUBJECTS STUDIED AT 6-HR INTERVALS

	1 pm				7 pm				1 am				7 am			
	PI				PI				PI				PI			
Subject	0	120 min	$T_{1/2}$	PIT	0	120 min	$T_{1/2}$	PIT	0	120 min	$T_{1/2}$	PIT	0	120 min	$T_{1/2}$	PIT
1	77	73	52	0.860	46	46	47	0.626	47	46	49	0.618	60	64	50	0.746
2	68	57	51	0.811	61	62	49	0.780	77	77	52	0.981	90	96	55	1.025
3	113	109	73	0.900	114	108	65	1.040	114	127	73	0.935	152	151	82	1.070
4	84	88	70	0.736	86	89	63	0.849	91	89	61	0.955	86	85	70	0.764
5	183	147	128	0.894	117	89	68	1.080	67	70	65	0.689	97	116	81	0.760
6	162	111	80	1.200	90	80	60	0.960	66	77	58	0.732	93	116	84	0.695
7	95	71	78	0.801	61	61	66	0.610	57	57	66	0.589	68	80	69	0.643
8	72	56	56	0.788	61	64	57	0.669	61	65	57	0.694	69	68	58	0.739
Mean	107		74	0.874	80		59	0.827	73		60	0.774	89		67	0.805

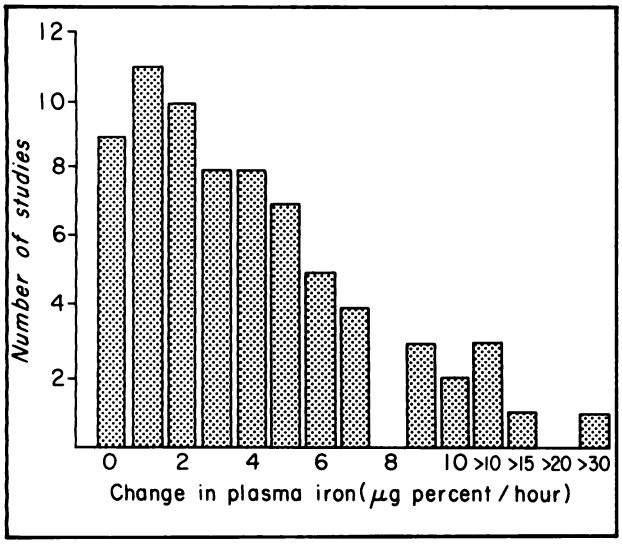

FIG. 1. Change in plasma iron during 120 min of subjects studied at 12-hr intervals.

The results of plasma iron-turnover measurements at 6-hr intervals are shown in Table 3. Paired analysis of turnover measurements at 6-hr intervals show p values greater than 0.05 and were not considered significant. Further calculations were made relating to plasma iron inflow and outflow (Table 4). Both inflow and outflow were changing, but no systematic changes were evident.

DISCUSSION

The measurement of plasma iron turnover calculated from the plasma iron level and initial rate of disappearance of radioiron appears to be a valid measurement of total erythropoiesis in normal subjects if allowance is made for iron reflux and iron localization in nonerythroid tissues (*1*). The mean value of 0.71 mg/100 ml of whole blood/day obtained in this study is similar to the composite mean of 0.7 of other reports in the literature (*8–18*). The variation of individual means obtained in our three studies of 0.62, 0.80 and 0.87 mg/100 ml whole blood/day was considerable, but was similar to variations reported by other authors whose mean values ranged from 0.58 to 0.85. Variations among the entire group of subjects which we have studied amounted to ±24% as compared with the individual variations in Groups 1 and 2 of ±12%. Since our studies were all carried out in the same laboratory with calibrated methods, it seems unlikely that laboratory error explains the differences in the three groups. While there is no direct evidence to bear on this group difference, it is suggested that changes in red-cell mass occur seasonally and that red-cell production may be expected to vary accordingly. The three studies reported here were carried out at different occasions.

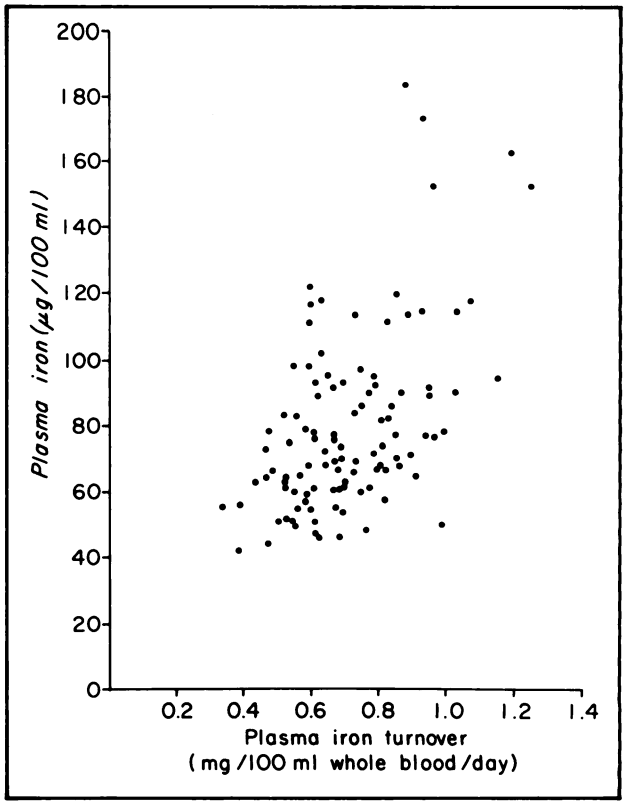

FIG. 2. Relationship of plasma iron to plasma iron turnover. Plasma iron for each single study is plotted against plasma iron turnover.

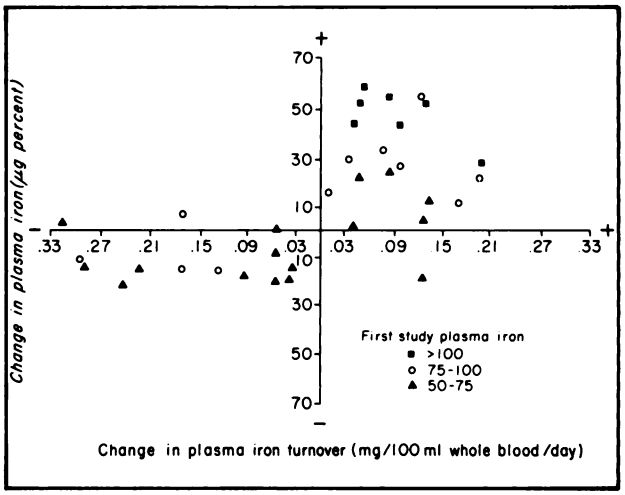

FIG. 3. Comparison of change in plasma iron with plasma iron turnover determined at 12-hr intervals. Symbols indicate plasma iron level of baseline study.

The attention of this study was directed at changes in plasma iron turnover which occurred within the 24-hr period. In order to minimize the effect of subject manipulation, two studies were carried out on each of two groups at different 12-hr intervals, i.e., 7 am and 7 pm, and 1 am and 1 pm. During these studies the plasma iron levels appeared to be

TABLE 4. PLASMA IRON OUTFLOW AND INFLOW IN SUBJECTS STUDIED AT 6-HR INTERVALS

	Measured outflow*				Measured change in PI*				Calculated inflow*			
Subject	1 pm	7 pm	1 am	7 am	1 pm	7 pm	1 am	7 am	1 pm	7 pm	1 am	7 am
1	62	40	40	50	− 5	− 1	+ 1	+ 3	57	39	41	53
2	55	52	62	68	−11	+ 2	+ 3	+ 1	44	54	65	69
3	64	73	65	77	− 4	0	+ 5	+ 1	60	73	70	78
4	50	57	62	51	+ 1	+ 1	− 1	− 1	51	58	61	50
5	59	72	43	50	−24	−10	0	+10	35	62	43	60
6	84	62	47	46	−24	− 4	0	+ 8	60	58	47	54
7	51	38	36	41	−14	− 1	0	+ 5	37	37	36	46
8	54	44	44	49	−10	+ 2	0	+ 1	44	46	44	50

* μg/100 ml plasma/hr

falling slightly at 7 am, 1 pm and 7 pm at rates of 1, 3 and 3 μg/hr, respectively. While such changes are difficult to measure and represent the means in a group with considerable individual variation, this rate of fall would be consistent with the diurnal variation of 30 to 40 μg/100 ml (*18*). At these four time intervals, inter-group comparisons of plasma iron-turnover values showed a mean decrease of 10% from 7 am to 7 pm, and of 11% from 1 pm to 1 am. Thus with a falling plasma iron, the plasma iron turnover also decreased.

The relationship between plasma iron level and plasma iron turnover has been further illustrated in Figs. 2 and 3 where, both among the group as a whole and individual subjects, the plasma iron level appears to bear a relation to the plasma iron turnover. It appears that a change in plasma iron of 10 μg/100 ml is associated with a plasma iron-turnover change of 0.03 mg/100 ml whole blood/day. Similar relationships but of much greater magnitude have been observed in subjects with hypoferremia (*19*). Here increases in plasma iron result in a proportional increase in plasma iron turnover. Loading studies in normal subjects where the plasma iron is increased to about 300 μg/100 ml have shown increases of about 0.3 μg/100 ml whole blood/day in the plasma iron-turnover (*20*). Thus it appears that the plasma iron turnover is influenced by levels of plasma iron, even with fluctuations within the normal range as demonstrated in the present study.

When repeated studies were carried out over the 24-hr period, there were no consistent changes in either plasma iron or plasma iron turnover over 6-hr intervals.

Certain general interpretations of the data obtained may be appropriate. It is evident that a state of dysequilibrium within the 24-hr period exists in the normal subject as reflected in the cyclic change in size of the plasma iron pool. In these studies there was no evidence that decrease is accomplished through a loss of iron from the plasma but rather that variations in loss reflect largely changes in the plasma iron level. The diurnal variation in plasma iron must then relate to a varying input of iron from the reticuloendothelial cell. This in turn may be related either to diurnal changes in the amount of red-cell destruction, since most of catabolized red-cell iron is reshunted to the plasma, or to changes in reticuloendothelial cell behavior. In addition, there are irregularities in plasma iron turnover which suggest some independence in behavior of both input and removal of iron from plasma. It is to be expected that changes in erythropoiesis may affect plasma iron turnover. Erythropoietin output is known to vary greatly from day to day and within the 24-hr period (*21*). It is also known that the erythroid marrow can respond to erythropoietin with an increase in plasma iron turnover within 24 hr (*22*). Thus one may visualize rapid oscillations in erythropoietin stimulation occurring within hours, slower changes in plasma iron turnover within 1 or 2 days and still slower changes of lesser magnitude in the total red-cell mass. The repeated studies in subjects of Group 3 suggest that there are independent changes in reticuloendothelial input and in removal presumably by the erythroid marrow. Even with these fluctuations, however, there is a remarkable degree of coordination between input and output since plasma iron changes usually occur at a rate of 3 or 4 μg/hr with a total turnover of approximately 60 μg/hr.

SUMMARY

Plasma iron turnover has been used extensively as a simplified measure of total erythropoiesis and in more complex estimates of internal iron exchange. Of concern to both is the question of stability of iron kinetics since all calculations are based on an equilibrium state. Past studies have shown differences in repeated plasma iron turnovers but no consistent

variation during the day. This has been examined in detail by repeated measurement over the 24-hr period in 45 normal subjects involving 106 determinations. The studies consisted of two groups studied at 12-hr intervals and one group at 6-hr intervals. Mean values of initial studies were 0.62, 0.80 and 0.87 mg/100 ml whole blood/day with a composite mean of 0.71. Variation among all subjects was ±24%. Plasma iron turnover changed in the direction of plasma iron at a rate of 0.036 mg/100 ml whole blood/day for a variation in plasma iron of 10 μg%.

In the subjects studied at 6-hr intervals, both inflow and outflow were changing but no systematic changes were evident. These studies indicate that cyclic change in the plasma iron pool relates primarily to varying inflow; and outflow is influenced by the plasma iron level. However, changes in turnover are small in comparison to variation in plasma iron.

ACKNOWLEDGMENTS

This investigation supported by U.S. Public Health Service Research Grant 5-R01-HE-06242 and Health Program Services Grant 0-69-3-66.

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