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FERROKINETIC STUDIES IN YOUNG CHILDREN : II. PLASMA IRON TURNOVER STUDIES IN HEALTHY CHILDREN.

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ABSTRACT

The plasma iron turnover is carried out in 5 healthy Thai Children, 1-4 year of age. The time that is required for half the original radioiron activity to disappeared from the plasma, the plasma radioiron disappearance half time ($T\frac{1}{2}$), is 68.90 ± 6.69 (range 61-75) minutes which is slightly shorter than those observed in adult subjects. The calculated plasma radioiron disappearance rate of 60.81 ± 5.91 (range 54-68) percent/hour is also in the high normal range of adult value of 35 to 70 percent/hour. The plasma radioiron turnover rate (PITR) of 1.33 ± 0.30 mg/100 ml whole blood /day and 0.98 ± 0.23 mg/Kg/day are also higher than the adult values. Our results strongly indicated that the iron turnover is more active in young children and that the observed findings in children with diseases must be compared against normal values of their age group for the correct interpretation.

INTRODUCTION

Exchange of iron between body tissues is considered to be accomplished by a plasma transport mechanism, involving the reversible binding of iron to transferrin or siderophilin [1]. Since the plasma represents the sole means of transport of iron from one site to another, measurements of iron turnover through this compartment have provided much of our understanding of

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both the normal and disordered erythron. The simplest calculation of iron turnover is based on calculation of the amount of iron leaving the plasma per unit time. Radioiron disappearence from plasma is considered to reflect turnover of transferrin-bound iron, since unbound iron is cleared too rapidly to affect the measurement. The rate of disappearance of radioiron from plasma is a function of the size of the plasma iron pool and the amount removed by tissues. This measurement has more value as the accurate indicator of total erythroid activity than as the clearance rate, since non-erythroid turnover normally is a minor fraction especially when iron exchange is above normal.

The plasma iron turnover (PIT) in which the daily transport of iron through the plasma is calculated from the plasma iron concentration and the initial rate of disappearance of transferrin-bound iron, has been extensively used as a highly useful quantitative measure of red cell production. In a large series of patients it has been demonstrated that the PIT is a valid indicator of total erythroid activity and provide the most accurate information in patients with accelerated red cell production.

At present, there is no published data on the plasma iron turnover study in young children available. We are reporting such data obtained from young Thai children as part of our study on the mechanism of anemia in malnutrition.

MATERIAL AND METHOD.

The format of the study was similar to those described by Huff et al[2] and Finch et al[3] as described in detail by Kulapongs et al[4]. Briefly, 2 ml of autologous plasma was incubated at 37°C with 0.2 mCi/Kg body weight of ^{59}Fe (as ferric citrate) then injected intravenously back into the subject. Blood samples were drawn after 10, 20, 30, 45 and 60 minutes for plasma volume and plasma ^{59}Fe disappearance curve determinations. The baseline plasma iron, total iron binding capacity as well as the routine hematologic parameters were determined prior to the injection.

CALCULATION.

N.K., a 2.75 year old Thai girl weighing 11.20 Kg. with a hemoglobin level of 12.4 gm/100 ml, hematocrit 35.5%, plasma iron level 88 mcg/100 ml, total iron binding capacity 286.5 mcg/100 ml is used as an example.

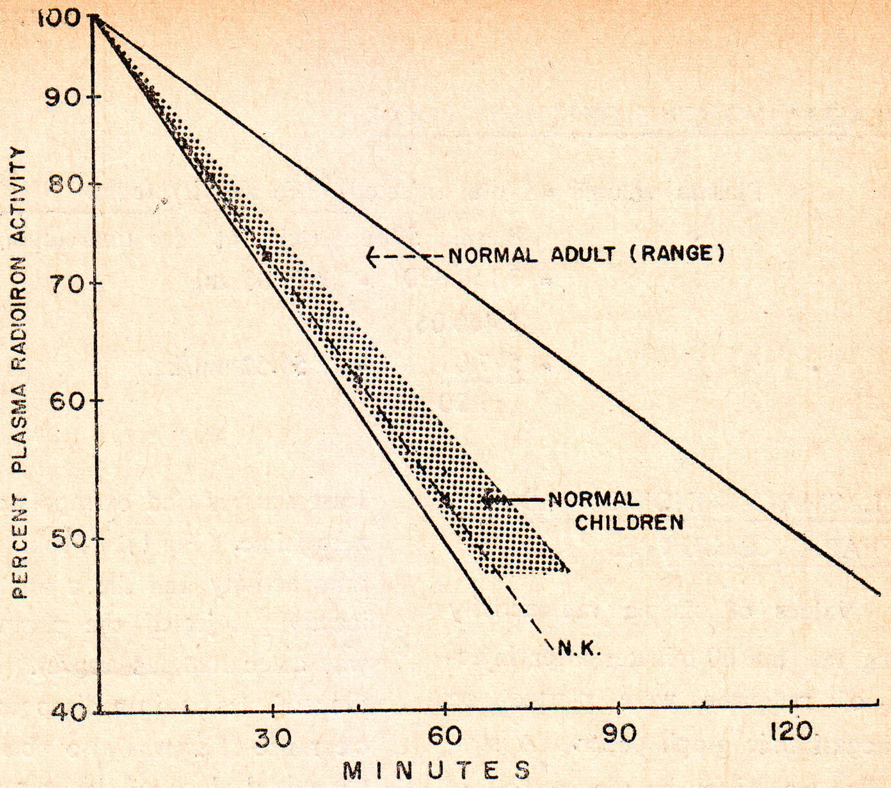

FIGURE I. PLASMA RADIOIRON DISAPPEARANCE CURVE OF THE SUBJECT (N.K.) AND THE NORMAL RANGE OF NORMAL CHILDREN AND ADULTS.

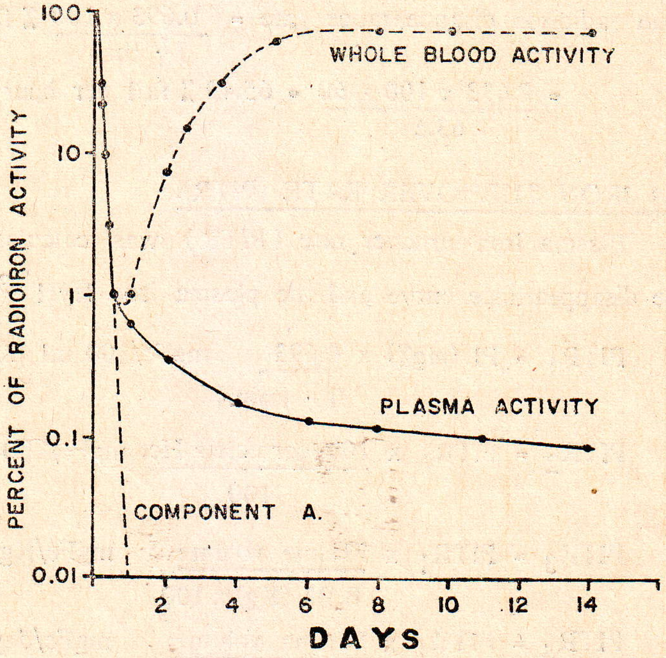

FIGURE II. PLASMA RADIOIRON DISAPPEARANCE CURVE AND WHOLE BLOOD RADIOACTIVITY.

The clearance rate progressively decreases with time. The initial clearance rate of iron by tissues is indicated by the component A.

1. PLASMA VOLUME DETERMINATION.

$$\text{Plasma volume} = \frac{\text{Total injected } ^{59}\text{Fe activity (cpm)}}{\text{Plasma } ^{59}\text{Fe activity at zero time (cpm/ml)}}$$

$$= \frac{3{,}150{,}620}{5{,}460.06} = 577.03 \text{ ml}$$

$$= \frac{577.03}{11.20} = 51.52 \text{ ml/kg.}$$

2. PLASMA RADIOIRON DISAPPEARANCE CURVE.

Values of plasma radioactivity during the first 60 minutes after injection of radioiron were plotted on semilogarithmic graph paper. A straight line was fitted by the method of least squares and extrapolated back to zero time. (Fig I). From this zero time activity and the amount of radioactivity injected, the plasma volume was calculated (as above). The half-time of plasma iron disappearance or clearance ($T\frac{1}{2}$) was also obtained from the initial slope of this curve.

3. PLASMA RADIOIRON DISAPPEARANCE RATE.

$$\text{Plasma radioiron disappearance rate} = \frac{0.693 \times 100}{T\frac{1}{2}\text{ (hr.)}} \text{ \% fall per hour}$$

$$= \frac{0.693 \times 100 \times 60}{63.5} = 65.49 \text{ \% fall per hour}$$

4. PLASMA IRON TURNOVER RATE (PITR).

Plasma iron turnover rate (PITR) was calculated from the initial slope of the disappearance curve and the plasma iron level (3, 5, 6).

$$PITR_1 = PI\text{ (mg\%)} \times \frac{0.693}{T\frac{1}{2}\text{ (min.)}} \text{ mgFe/100 ml plasma/day}$$

$$PITR_2 = PITR_1 \times \frac{100\text{-corrected Hct}}{100} \text{ mgFe/100 ml whole blood/day}$$

$$PITR_3 = PITR_1 \times \frac{\text{Plasma volume}}{\text{B.W. (Kg)} \times 100} \text{ mgFe/Kg/day}$$

$$PITR_4 = PITR_1 \times \frac{\text{Plasma volume}}{100} \text{ mgFe/day}$$

Here, 0.693 is the natural logarithm of 2; 1,440 is the minutes in 24 hours; PI is the plasma iron level; and the corrected Hct. is the observed hematocrit x 0.96 x 0.92 (0.96 is the correction for trapped plasma volume and 0.92 is for the mean body hematocrit). For our patient N.K.,

$$PITR_1 = \frac{.088 \times 1440 \times 0.693}{63.5} = 1.38$$

$$PITR_2 = \frac{1.38 \times 100 - 31.0}{100} = 0.95$$

$$PITR_3 = \frac{1.38 \times 577}{11.2 \times 100} = 0.71$$

$$PITR_4 = \frac{1.38 \times 577}{100} = 7.98$$

5. PLASMA IRON POOL (PIP) (6).

$$PIP = \text{Plasma iron (mg/ml)} \times \text{Plasma volume}$$

$$= \frac{0.88 \times 577}{1,000} = 0.507 \text{ mg.}$$

RESULTS.

The hematologic and nutritional status of 5 healthy children studied are shown in TABLE I. The results of various parameters of iron turnover are shown in TABLE II.

TABLE I. : HEMATOLOGIC AND NUTRITIONAL STATUS OF SUBJECTS

	S.T.	J.J.	U.C.	N.K.	C.P.	MEAN ± S.D.
Age (yr.)	2.00	1.50	1.50	2.75	4.00	
Body Weight (Kg.)	11.24	7.26	6.88	11.20	8.74	
Hb. (gm/100 ml.)	12.70	12.30	11.40	12.40	12.50	12.26 ± 0.50
Hct. (%)	39.00	35.00	36.00	35.50	38.00	36.70 ± 1.72
Plasma Iron (mcg/100 ml.)	172.00	114.00	189.50	88.00	121.00	136.90 ± 42.33
TIBC (mcg/100 ml.)	321.00	292.50	337.50	286.50	330.00	313.50 ± 28.77
TSP. (gm/100 ml.	7.60	7.85	7.20	7.45	7.80	7.58 ± 0.26
Albumin (gm/100 ml.)	3.94	4.14	4.04	3.81	4.00	3.98 ± 0.10
Globulin (gm/100 ml.)	3.66	3.71	3.16	3.64	3.80	3.59 ± 0.24

TABLE II: RESULTS OF THE PLASMA IRON TURNOVER STUDY IN HEALTHY CHILDREN

PARAMETERS	S.T.	J.J.	U.C	N.K.	C.P.	MEAN ± S.D.	ADULT VALUES
Plasma iron (mcg/100ml)	172.00	114.00	189.50	88.00	121.00	136.90 ± 43.33	
Plasma volume : (ml)	508.00	399.00	367.00	577.00	402.00		
: (ml/Kg)	45.20	55.00	53.40	51.50	46.00	50.22 ± 4.40	
Plasma Iron Pool : (mg)	0.87	0.45	0.70	0.51	0.49	0.60 ± 0.17	3.50 (2.30–4.60)
: (mg/Kg)	0.077	0.063	0.101	0.045	0.056	0.068 ± 0.021	0.05 (0.033–0.066)
Plasma Radioiron Dis-appearance $T\frac{1}{2}$ (min)	76.0	69.00	75.0	63.50	61.00	68.90 ± 6.70	60–120
PRD RATE (% perhour)	54.71	60.26	55.44	65.49	68.16	60.81 ± 5.96	35–70
PLASMA RADIOIRON TURNOVER RATE (PITR)							
: $PITR_1$ (mg/100 ml plasma/day	2.26	1.65	2.52	1.38	1.98	1.96 ± 0.45	1.05 (0.8–1.1)
: $PITR_2$ (mg/100 ml whole blood/day)	1.49	1.14	1.73	0.95	1.32	1.33 ± 0.30	0.61 (0.38–0.77)
: $PITR_3$ (mg/Kg/day)	1.02	0.91	1.34	0.71	0.91	0.98 ± 0.23	0.56 (0.46–0.78)
: $PITR_4$ (mg/day)	11.47	6.58	9.25	7.96	7.98	8.65 ± 1.84	37 (27–42)

COMMENTS.

The time that is required for half the original radioiron activity to disappeared from the plasma is called the plasma radioiron disappearance half time ($T\frac{1}{2}$) and in normal adult subject is 60 - 120 minutes. The results obtained from 5 healthy Thai children in our study revealed a $T\frac{1}{2}$ value of 68.90 ± 6.69 (61-75) minutes which is slightly shorter than those of adult subjects (3, 6-8). The observed plasma radioiron disappearance rate of 60 81 ± 5.96 (54-68) % per hour in these children is in the high normal range of the adult value of 35-70 % per hour. It is generally recognized that the plasma radioiron disappearance (PRD) or clearance rate is linear only for the first few hours. Our results confirmed the observations made earlier by Sharney et al [9] and Garby et al [10] that the disappearance curve is a single exponential only in its first 3/4 hour, where upon a slower rate becomes evident. Normally, there is a true exponential disappearance of radioiron activity from the plasma until about 90 % of the original activity is gone. If the PRD curve does not remain exponential until at least 80-90% of the initial activity has left the plasma, serious errors may be introduced in calculations of the erythropoietic activity. After this the slope of the curve changes because of iron reflux from labile pool, and the clearance no longer reflects iron uptake alone.

It has been appeared for some time that the PRD curve is a complex function not adequately portrayed by simple formulation. In normal adult, the slope of PRD curve changes from about 40% per hour initially to less than 1% per hour after the second day [3]. This apparent change in clearance has been shown to be due to the reflux of radioiron back into plasma [11]. Results of several studies of the amount of iron reflex in adult indicate a reflux of about 35% in normal subject [3, 7, 8]. More detailed analysis of PRD curve disclosed two recognizable exponential components. A rapid reflux of 5 - 10 % with a $T\frac{1}{2}$ of about 8 hours and a slow reflux of 25% with a $T\frac{1}{2}$ of about 8 days [3]. The more rapid reflux coincides in time and amount with the extravascular flux of the transferrin iron complex [12] and does not contribute significantly to total plasma iron turnover even in pathological states [7]. The slow reflex represents the wastage iron of erythropoiesis and is virtually about in aplastic anemia.

Although there is a general correlation between PRD $T\frac{1}{2}$ and erythropoietic activity the PRD rate is not a direct measure of erythropoietic tissue because it is dependent on the size of the circulating plasma iron pool and the amount removed by tissues. The PRD value is fast if the plasma iron level is low or the plasma volume is smaller, and slow if the plasma iron level is high or the plasma volume is expanded. Nevertheless, prolongation of the PRD or clearance is a very useful indication of decreased bone marrow activity. Since the normal plasma iron turnover (PIT) is low, a decrease may be difficult to recognize, whereas a prolongation of the PRD or clearance rate is easily measured. When PRD rates are very rapid ($T\frac{1}{2}$ of shorter than 30 minutes), it can be assumed that extraction of iron from plasma by the tissues is nearly maximal and that the supply to the marrow is suboptimal. When there is a marked reduction in the erythroid marrow mass, a very slow rate of clearance ($T\frac{1}{2}$ of over 3 hours) are found for extraction of transferrin iron by tissues other than the erythroid marrow and placenta is very limited.

The simplest calculation of iron turnover is based on determination of the amount of iron leaving the plasma per unit time. In such a calculation plasma iron is assumed to be homogeneous pool, turnover is calculated from the plasma iron level and the initial disappearance rate of radioiron, and the system is considered to be a steady state. If the level of stable iron in the plasma is constant, which is not correct (3) and if the disappearance of ^{59}Fe reflects removal of stable iron, which is correct if the iron-binding protein has been overloaded, then one could extend the disappearance rate of radioiron to turnover of stable iron. The value of plasma iron turnover (PIT) also includes a fraction of iron which leaves the plasma and returns after a few hours or days (reflux iron). It is therefore a more accurate measure of erythroid activity than PRD rate. To make results more comparable between individuals of different sizes the PIT can be related to weight. A simpler way of comparing individuals involves relating the PIT to 100 ml of whole blood, since it might be required to provide for the red cell needs of 100 ml of whole blood. If all iron cleared from plasma is used in the synthesis of hemoglobin, the normal adult values would be about 0.38 mg/100 ml whole blood/day. The observed normal value is higher because certain portion of iron cleared from the circulation is directed into parenchymal tissues and into the labile, nonerythropoietic pools. Therefore, its limitations are that it does not differentiate between erythroid and non-erythroid uptake (essential when iron exchange is depressed) and even more importance, it does not distinguish between marrow activity resulting in viable red cells (effective erythropoiesis) and that which does not (ineffective erythropoiesis). At any rate, in clinical situation it has been well demonstrated that PIT is a valid indicator of total erythroid activity. In states of marrow hyperfunction it is increased from 3 to 6 times normal and is depressed to approximately half the normal value in marked marrow hypofunction. In patients with hypoplastic marrow, the PRD rate may be a more useful measure than PIT value. It is important to realized that when PIT is expressed in relation to a fixed volume of blood (100 ml), one must be certain that the total blood or pasma volume bears a constant relationship to body size or body weight. Under condition of severe dehydration or hydremia, the PIT expressed in mg/100 ml whole blood / day may give misleading information about the size of the erythropoietic tissue. Our results strongly indicated that normal values must be established in children of different ages due to the variations in hemoglobin level, erythroid activity, body weight, blood and plasma volumes.

ย่อเรื่อง

คณะผู้วิจัยได้ทำการศึกษาเกี่ยวกับ Plasma iron turnover ในเด็กไทยที่สุขภาพสมบูรณ์ อายุระหว่าง 1-4 ปี จำนวน 5 คน ปรากฏผลว่าระยะเวลาที่ปริมาณของ Radioiron Activity ในพลาสม่าลดลงเหลือครึ่งหนึ่งของปริมาณเมื่อฉีดตอนแรก หรือที่เรียกว่า plasma radioiron disappearance half time ($T\frac{1}{2}$) คือ 68.90 $\pm$ 6.69 (ค่าอยู่ระหว่าง 61-75) นาที ซึ่งจะมีระยะเวลาสั้นกว่าที่ได้จากการทดสอบในผู้ใหญ่เล็กน้อย

ผลที่ได้จากการ คำนวณของ อัตราการ ลดลงของ Radioiron จากพลาสม่า คือ 60.81 $\pm$ 5.91 (ค่าอยู่ระหว่าง 54-68) เปอร์เซ็นต์ต่อชั่วโมง ซึ่งสูงกว่าค่าปกติในผู้ใหญ่คือ 35-70 เปอร์เซ็นต์ต่อชั่วโมง นอกจากนี้ค่า plasma radioiron turnover rate (PITR) ที่คำนวณได้คือ 1.33 $\pm$ 0.30 มก. ต่อเลือด 100 มล. ต่อวัน และ 0.98 $\pm$ 0.23 มก. ต่อ ก.ก. ต่อวัน ซึ่งก็เป็นค่าที่สูงกว่าค่าที่ได้จากผู้ใหญ่ปกติ

ผลที่ได้จากการศึกษาครั้งนี้จึงช่วยแสดงให้เห็นอย่างชัดเจนว่า Iron turnover ในเด็กจะ active กว่าในผู้ใหญ่ปกติ และค่าที่ได้เหล่านี้ในเด็กผู้ป่วย ควรจะนำมาเปรียบเทียบกับค่าปกติในเด็กกลุ่มที่มีอายุเท่า ๆ กัน เพื่อให้ได้ผลการวิเคราะห์ที่ถูกต้องยิ่งขึ้น.

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