



FERROKINETIC STUDIES IN YOUNG CHILDREN: III. RED CELL UTILIZATION, RED CELL IRON TURNOVER AND ERYTHRON TURNOVER STUDY.

By

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INTRODUCTION:

It is unfortunate that no single ferrokinetic procedure can be relied on to provide unequivocal information regarding the state of erythropoiesis in any given situation. As the radioiron enters the bone marrow it is incorporated into newly formed red cells, and a progressive increase in radioactivity appears in the blood as labeled red cells enter the circulation. When radioiron is injected intravenously in a normal subject, it is estimated that 85% of the radioactivity goes directly to the marrow while 15% goes first to iron storage areas (liver and spleen), to be released later for red cell produc-

tion. The sum total of this movement of iron results in the appearance of 75 to 90% of the injected radioiron in circulating red cells within 2 weeks. The average radioiron incorporation or utilization is approximately 80% in 7 to 10 days. The determination of radioiron red cell utilization has been shown to be a valuable measurement of "effective" erythropoiesis. Although it is difficult to make a quantitative estimation of red cell production from this test alone, it generally can be relied on to detect reduced red cell production in instances when the marrow morphology may be misleading.

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In order to calculate the amount of iron going to the circulating red cells, it is necessary not only to determine the plasma iron turnover (PIT) but also the distribution of radioiron between red cells and other tissues at 2 weeks. A convenient measure of effective red cell production is the red cell iron turnover. It records only that fraction of iron utilized in the synthesis of viable mature red cells. Theoretically, the red cell iron turnover rate should be a more accurate quantitative measurement of effective erythropoiesis than the PIT rate. A calculation of red cell iron turnover rate (RCITR) or erythrocyte iron turnover (EIT) was also devised by Huff et al. (1). Here, the plasma iron turnover (PIT) was divided into a red cell portion (erythron turnover) and non-red cell portion (non-erythron turnover) according to the amount of radioiron present in the red cell mass at 10 to 14 days. It is possible to assess the erythron turnover by subtract from the PIT, the non-erythron turnover which can be computed from the plasma iron level, plasmacrit, and a constant (0.0035) (2-3). The erythron turnover value has been claimed

to be a highly quantitative measurement of the number of nucleated red cells in the marrow and their hemoglobin synthesizing capacity (3)

We are presenting our results of the detail study of the red cell utilization, red cell iron turnover and erythron turnover in healthy Thai children in Chiang Mai area.

MATERIAL AND METHOD.

The format of the study was similar to those described by Huff et al. (1) and Finch et al. (2) as reported in detail by Kulapongs et al. (4,5). Five healthy young children, 1.5 to 4 years of age, were studied.

CALCULATIONS.

C.P., a 4 year old Thai girl weighed 8.74 Kg. with a hemoglobin level of 12.5 gm/100 ml, hematocrit 38%, plasma iron level of 121 mcg/100 ml with TIBC 330 mcg/100 ml and the plasma iron turnover PIT value of 0.98 mg/100 ml whole blood/day, 5.91 mg/day or 0.676 mg/Kg/day is used as an example.

1. BLOOD VOLUME AND RED CELL MASS.

$$\begin{aligned} \text{Blood volume} &= \frac{\text{Plasma volume} \times 100}{100 - \text{corrected Hct}} \\ &= \frac{402 \times 100}{100 - (38 \times 0.96 \times 0.91)} = 602 \text{ ml.} \end{aligned}$$

$$\begin{aligned}\text{Red cell mass} &= \text{Blood volume} - \text{Plasma volume} \\ &= 602 - 402 = 200 \text{ ml. or } 22.88 \text{ ml/Kg.}\end{aligned}$$

II. TOTAL RED CELL IRON.

$$\begin{aligned}\text{Total red cell iron} &= \text{Blood volume (ml)} \times \frac{\text{Hb(gm/100ml)}}{100} \times 3.4 \\ &= 602 \times \frac{12.5}{100} \times 3.4 \\ &= 255.85 \text{ mg total or } 29.27 \text{ mg/Kg.}\end{aligned}$$

Here, 3.4 is the amount of iron (mg) in 1 gm. of hemoglobin.

III. RED CELL UTILIZATION. (RBCU)

The red cell utilization of radioiron expressed as a percent of the injected activity is simply calculated by comparing the highest blood activity (between 10-14 days)

with the zero time activity. This calculation is dependent on the accurate determination of the blood activity constant blood volume and the adopted value of the mean body hematocrit (0.91).

$$\begin{aligned}\text{RBCU} &= \frac{14 \text{ days activity/ml blood} \times \text{Blood volume} \times 100}{\text{Total injected activity}} \\ &= \frac{2,311 \times 602 \times 100}{1,767,589} = 78.70 \%\end{aligned}$$

IV. RED CELL IRON TURNOVER RATE (RCITR) OF ERYTHROCYTE IRON TURNOVER (EIT)

Red cell iron turnover indicates fraction of iron utilized by bone marrow erythroid cells for hemoglobin synthesis.

$$\begin{aligned}\text{RCITR} &= \text{PIT} \times \text{maximal red cell utilization} \\ &= 1.32 \times \frac{78.70}{100} \\ &= 1.039 \text{ mg/100 ml blood/day.} \\ \text{or RCITR} &= 7.96 \times \frac{78.70}{100} \\ &= 6.265 \text{ mg/day} \\ \text{or} &= 0.717 \text{ mg/Kg/day.}\end{aligned}$$

TABLE 1: RED CELL UTILIZATION, RED CELL IFCN TURNOVER AND IRYTHRON TURNOVER IN HEALTHY CHILDREN.

| | S.T. | J.J. | U.C. | N.K. | C.P. | $\bar{x} \pm S.D.$ | ADULT VALUES (2,3,6.) |
|---------------------------|--------|--------|--------|--------|--------|--------------------|-----------------------|
| AGE (yr.) | 2.0 | 1.5 | 1.5 | 1.75 | 4.0 | | |
| 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 \pm 0.50 | |
| Hct. (%) | 39 | 35 | 36 | 35.6 | 38 | 36.70 \pm 1.72 | |
| PLASMA IRON (mcg/100 ml) | 172 | 114 | 189.5 | 88 | 121 | 136.90 \pm 42.33 | |
| TIBC (mcg/100 ml) | 321 | 292.5 | 337.5 | 286.5 | 330 | 313.50 \pm 22.77 | |
| BLOOD VOLUME (ml) | 771 | 575 | 535 | 836 | 602 | | |
| RED CELL MASS: (ml.) | 263 | 176 | 168 | 259 | 200 | | |
| : (ml/kg) | 23.4 | 24.2 | 24.4 | 23.1 | 22.88 | 23.59 \pm 0.67 | |
| TOTAL RED CELL IRON (mg) | 332.92 | 240.47 | 207.37 | 352.46 | 255.85 | 277.81 \pm 62.15 | |
| RED CELL UTILIZATION (%) | 80.54 | 92.98 | 90.93 | 73.31 | 78.70 | 83.29 \pm 8.37 | 80 (74 - 86) |
| PLASMA IRON TURNOVER RATE | | | | | | | |
| : mg/100 ml blood/day | 1.49 | 1.14 | 1.73 | 0.95 | 1.32 | 1.32 \pm 0.30 | 0.70 (0.68 - 0.85) |
| : mg/day | 11.47 | 6.58 | 9.25 | 7.98 | 7.96 | 8.64 \pm 1.84 | |
| RED CELL IRON TURNOVER | | | | | | | |
| : mg/100 ml blood/day | 1.20 | 1.06 | 1.57 | 0.70 | 1.04 | 1.11 \pm 0.30 | 0.56 (0.3 - 0.7) |
| : mg/day | 9.238 | 6.118 | 8.411 | 5.850 | 6.265 | 7.176 \pm 1.54 | 29 (20 - 39) |
| : mg/kg/day | 0.822 | 0.843 | 1.223 | 0.522 | 0.717 | 0.825 \pm 0.255 | 0.52 (0.43 - 0.72) |
| RBC IRON RENEWED (%/day) | 2.78 | 2.54 | 4.06 | 1.66 | 2.45 | 2.69 \pm 0.87 | 1.3 (0.9 - 1.8) |
| NONERYTHRON TURNOVER | | | | | | | |
| (mg/100 ml blood/day) | 0.397 | 0.277 | 0.455 | 0.213 | 0.283 | 0.325 \pm 0.095 | 0.15 \pm 0.001 |
| ERYTHRON TURNOVER | | | | | | | |
| (mg/100 ml blood/day) | 1.093 | 0.863 | 1.275 | 0.737 | 1.037 | 1.001 \pm 0.207 | 0.60 \pm 0.02 |

V. PERCENT RBC IRON RENEWED PER DAY.

The amount of iron utilized by bone marrow erythroid cells for hemoglobin synthesis can be expressed as fraction of total red cell iron as follow:

$$\begin{aligned}\text{RBC Iron renewed} &= \frac{\text{RCITR (mg/day)}}{\text{Total RBC iron (mg)}} \times 100 \\ &= \frac{6.265 \times 100}{255.85} = 2.45 \% \text{ per day.}\end{aligned}$$

VI. ERYTHRON TURNOVER.

The erythron turnover value is calculated by subtracting the non-erythron turnover from the plasma iron turnover.

$$\begin{aligned}\text{ET} &= \text{PIT} - \text{plasma iron (mcg/ml)} \times \text{Plasmacrit} \times 0.0035 \\ &= 1.23 - \left(\frac{121}{100} \times (100 - 33.19) \right) \times 0.0035 \\ &= 1.32 - 0.283 \\ &= 1.037 \text{ mg iron/100 ml. Blood/day.}\end{aligned}$$

RESULTS.

Results of the study in 5 healthy children is tabulated in Table I.

COMMENTS.

The utilization curve portrays the appearance of radioiron in the circulating red cell mass. In normal adult subjects (2,7) and our normal children, about 80% of injected iron is found in the circulating blood at 14 days. Usually there is a delay of approximately 1.7 days and then an exponential release of radioiron from the marrow with a $T_{1/2}$ of about 1.8 days. Since the radioiron is fixed within a few hours within the erythroid marrow as hemoglobin, the lag phase represents the time required for labeled cells to mature and released into the circulation.

Because the normal red cell utilization of radioactive iron may be as high as 90%, a further increase has little significance. A decrease utilization, however, is an important finding and suggests that mature red cell are destroyed shortly after their release from the bone marrow (hemolysis), that immature red cells are destroyed in the bone marrow before their release to the circulation (ineffective erythropoiesis), or that serum iron, because of slow bone marrow uptake, is diverted to non-erythropoietic tissues (bone marrow hypoplasia). Severe peripheral hemolysis can be recognized from the shape of RBCU curve which

displays an early rise (shortened marrow transit time), an early maximum utilization, and a subsequent fall-off (FIG.III). Ineffective erythropoiesis is characterized by a shortened radioactive iron disappearance rate and bone marrow hypoplasia by a prolonged radioactive iron disappearance rate. In ineffective erythropoiesis most of the radioactive is never seen in the circulating red cell mass. One of the most direct way to examine the meaning of the utilization curve is to determine the uptake distribution of radiation among the erythroid cells of the bone marrow and to derive from other parameter of erythropoiesis the time required for these labeled cells to appear in the circulating blood.

Calculation of erythrocyte iron turnover (EIT) or red cell iron turnover rate (RCITR) is the product of plasma iron turnover (PI Γ) x red cell utilization (RBCU) and is, therefore, influenced by the manner of calculation of each. In addition to the correction factor for trapped plasma volume, another fixed correction factor for the mean body hematocrit is involved. The former is invalid in patients with hypochromic and/or abnormal shape red cell, while the latter correction factor can not be used in splenome-

galy. In any patient whose mean body hematocrit may be altered, such as in splenomegaly, it is necessary to determine red cell mass separately with the ^{51}Cr -tagged red cell technique. In "ineffective" erythropoiesis and in "hemolytic anemias" the RCITR value underestimates effective erythropoiesis⁽³⁾. In these conditions red cell utilization is depressed for 2 different reasons: (1) in hemolytic anemias the continuous turnover of radioiron through the erythron-reticuloendothelial circuit results in a uniform specific activity of its iron and utilization will be depressed proportionate to the radioiron held in the erythroid marrow and RE cells, and⁽²⁾ in "ineffective" erythropoiesis, the utilization curve has not yet reached an equilibration state at 2 weeks, since most of the radioiron is still cycling between erythroid marrow and RE cells. While the RCITR value can not be considered to have quantitative meaning in patients with hemolytic anemia or ineffective erythropoiesis, it can be used as a rough indication of the efficiency of erythropoiesis.

A convenient and more precise index of effective red cell production is the erythron iron turnover since

it records only that fraction of iron utilized in the synthesis of viable mature red cells. Theoretically both the RCITR and erythron iron turnover rate should be the more accurate quantitative measurements of effective erythropoiesis than the PIT rate. But these tests are also dependent upon the measurement of plasma disappearance rate and PIT and hence are limited by the errors inherent in those determinations. At any rate, the erythron turnover value has been found to be a highly quantitative measurement of the number of nucleated red cells in the marrow and their hemoglobin synthesizing capacity(3)

Results of our study indicated the higher iron turnover rate in young children than adult subjects, confirmed the results obtained from the plasma radioiron turnover study.

SUMMARY.

Detailed study of the kinetic of the injected radioactive iron (^{59}Fe) in the body is carried out in 5 healthy young children. The results of red cell utilization study indicated that $83.29 \pm 8.37\%$ of injected radioiron is taken up by

the hemopoietic and storage tissues and later incorporated into the hemoglobin of circulating red cell mass. Theoretically the effectiveness of bone marrow erythropoiesis can be more accurately estimated by the determination of red cell iron turnover rate (RCITR) or erythrocyte iron turnover (EIT.) From the present study it was found that the average RCITR in our children is 1.11 ± 0.30 mg/100 ml whole blood/24 hour or 0.825 ± 0.255 mg/Kg body weight/24 hour which slightly higher than those of the adult subject. The more important parameter is the erythron turnover rate which has been found to be a highly quantitative measurement of the number of erythroid precursor and their hemoglobin synthesizing capacity. The erythron turnover rate in our children is 1.001 ± 0.207 mg/100 ml. whole blood/24 hour which is also higher than the adult value. Results of the study indicated that in normal situation iron turnover is higher in young children and that those observed in diseased children should be compared with these values rather than the adult's values in literature.

ย่อเรื่อง

คณะผู้วิจัยได้ทำการ ศึกษาเกี่ยวกับรายละเอียด Kinetic ของ Radioactive Iron (^{59}Fe) ที่ฉีดเข้าไปในร่างกายของเด็กไทยที่มีสุขภาพสมบูรณ์จำนวน 5 คน ผลที่ได้จาก Red cell Utilization แสดงให้เห็นว่าปริมาณของ Radioiron ที่ฉีดเข้าไปจำนวน $83.29 \pm 8.37\%$ จะถูกจับโดย Hemopoietic และ Storage Tissues ซึ่งต่อมาจะเข้าไปรวมตัวกับ Hemoglobin ของเม็ดเลือดแดงในวงจรโลหิต

ตามทฤษฎีนั้นขีดความสามารถของ Bone Marrow Erythropoiesis จะวัดได้อย่างแม่นยำด้วยการตรวจหา Red Cell Turnover Rate (RCITR) หรือ Erythrocyte Iron Turnover (EIT) ซึ่งจากการศึกษาในครั้งนั้นพบว่าค่าเฉลี่ย RCITR ในเด็ก คือ $1.11 \pm 0.30 \text{ mg}/100 \text{ ml}/\text{Whole blood}/24 \text{ ชั่วโมง}$ หรือ $0.825 \pm 0.255 \text{ mg}/\text{Kg น้ำหนักตัว}/24 \text{ ชั่วโมง}$ ซึ่งจะสูงกว่าค่าที่ได้ในผู้ใหญ่เล็กน้อย

เครื่องชี้บ่งที่สำคัญอีกอันก็คือ Erythron Turnover Rate ซึ่งพบว่าเป็นปริมาณที่สามารถวัดได้ของจำนวน Erythroid Precursor และความสามารถที่จะสังเคราะห์ Hemoglobin ได้ ซึ่งมีปริมาณ Erythron Turnover Rate ที่

ตรวจได้ในเด็กครั้งนี้คือ $1.001 \pm 0.207 \text{ mg}/100 \text{ ml Whole blood}/24 \text{ ชั่วโมง}$ ซึ่งก็สูงกว่าค่าที่ได้จากผู้ใหญ่ด้วย

ผลที่ได้จากการศึกษาวิจัยครั้งนี้ จึงช่วยให้เห็นว่าในภาวะปกติ Iron Turnover ในเด็กจะมีค่าสูงกว่าผู้ใหญ่ อย่างไรก็ตามก็น่าที่จะได้ศึกษาเกี่ยวกับเรื่องนี้ในเด็กผู้ป่วย เพื่อที่จะได้นำค่ามาเปรียบเทียบกับค่าเด็ก ปกติ มากกว่าที่จะนำไปเปรียบเทียบกับค่าที่ได้ในผู้ใหญ่

REFERENCES

1. Huff, R.L., Elmlinger, P.J., Garcia, J.F., Oda, J.M., Cockrell, M.C., and Lawrence, J.H.: Ferrokinetics in normal persons and in patients having various erythropoietic disorders. J. Clin. Invest. 30: 1512, 1951.
2. Finch, C.A., Deubelbeiss, K., Cook, J.D., Eschbach, J.W., Harker, L.A., Funk, D.D., Marsaglia, G., Hillman R.S., Slichter, S., Adamson, J.W., Ganzoni, A., and Giblett, E.R.: Ferrokinetics in man. Medicine 49: 17, 1970.
3. Cook, J.D., Marsaglia, G., Eschbach, J.W., Funk, D.D., and Finch, C.A.: Ferrokinetics: a biological model for plasma iron exchange in man. J. Clin. Invest. 49: 197, 1970.

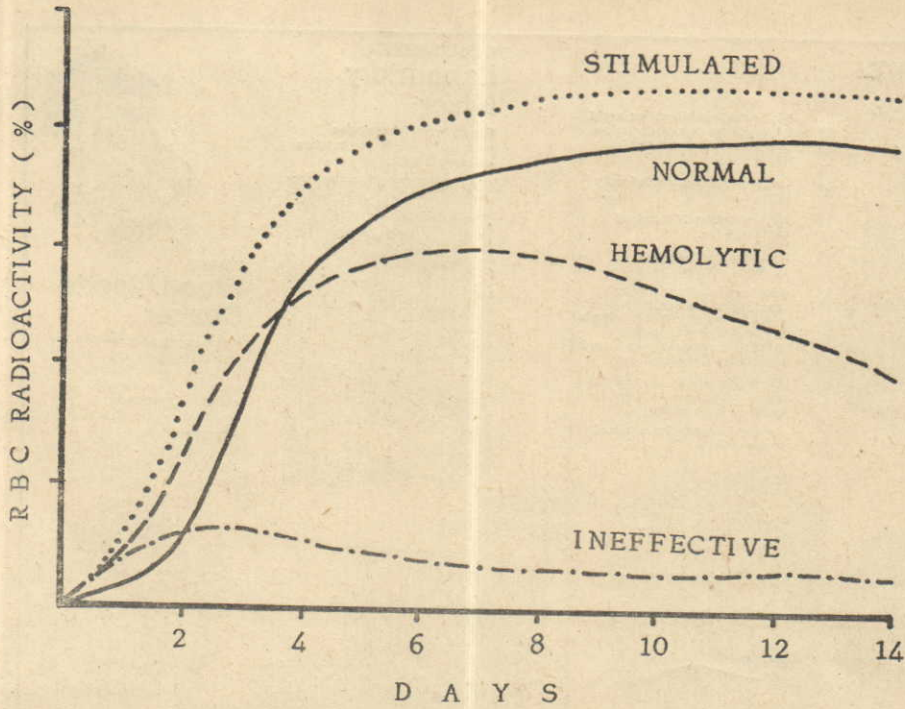


FIGURE III. RED CELL UTILIZATION CURVE :

In normal person a maximum utilization is reached in 2 weeks. With increased stimulation of the marrow, the appearance of radioiron in red cell is accelerated. In hemolytic anemias the curve displays an early rise, early maximum utilization and a subsequent early fall-off within 2-weeks. Ineffective erythropoiesis is characterized by a flat curve since most of the radioactivity is never seen in the circulating red cell mass.

4. Kulapongs, P., Thumapukgoon, K., and Tositarat, T.: Ferrokinetic studies in young children: I. Quantitation of total bone marrow cellularity in vivo by radioiron technique. *Bull. C.M. Assoc. Med. Sci.* 8: 63, 1975.
5. Kulapongs, P., Boonpala, O., and Tositarat, T.: Ferrokinetic studies in young children: II. Plasma iron turnover studies in healthy children. *Bull. C.M. Assoc. Med. Sci.* 9: 43, 1976.
6. Figueroa, W.G., and Weinstein, I.M.: Erythropoietic measurements with radioiron. In *Mechanism of Anemia*. Weinstein, I.M., and Beutler, E., Eds. The McGraw-Hill Book Co., Inc., New York, 1962.
7. Hosain, F., Marsaglia, G., and Finch, C.A.: Blood ferrokinetics in normal man. *J. Clin. Invest.* 46: 1, 1967.