The Measurement of Blood Loss and Its Effect on Red Cell Survival Studies with ^{51}Cr*

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=國 文 抄 錄=

失血이 赤血球壽命 測定에 미치는 影響에 關한 硏究

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赤血球 수명의 측정에는 ^{51}Cr-標識赤血球法이 臨床的으로 利用되고 있으며 이는 理論上 steady state 即 測定期間동안 循環 ^{51}Cr量-赤血球量이 一定할 때에 限하여 有效하며 unsteady state 때는 true red cell survival을 알기 위하여서는 측정치에 영향을 주는 要因에 대하여 各各 校正해 줄 必要가 있다.

이 要因중에 특히 失血로 因한 영향에 關하여는 계통적인 硏究가 적다. 이에 著者들은 ^{51}Cr-標識赤血球法을 利用하여 失血이 赤血球 수명測定에 미치는 영향을 人體에서 실험 관찰하여 몇가지 성적을 얻었다.

硏究對象은 總 56名의 靑壯年으로 急性失血群과 慢性失血群으로 區分하여 急性失血群은 胃腸出血等이 없는 20代의 醫大生으로 ^{51}Cr-標識赤血球法을 사용하여 赤血球 수명을 측정하는 동안(10~14日間) 1日當 10 ml(6名), 25 ml (4名), 50 ml (4名), 75 ml (4名), 100 ml (6名)를 各各 瀉血한 群과 10日間 1,000 ml를 사혈한 群 즉 200 ml씩 5回 (4名), 500 ml씩 2回 (4名)로 細分하였으며 慢性失血群은 직업적인 供血者로 반복사혈로 생긴 9名의 빈혈자와 十二指腸虫症에 感染되어 驅虫한 中等度의 철결핍성 빈혈환자 7名으로 나누어 관찰하였다.

測定 方法으로는 Gray 및 Sterling法을 개선한 方法으로 ^{51}Cr-標識赤血球의 計測試料로서 全血 및 赤血球를 使用하였다.

實驗성적은 1. 1日當 失血量이 增加할수록 赤血球수명($T_{\frac{1}{2}}$)은 짧아짐을 알 수 있었다.

即 1日當 20~50 ml 사혈군에서는 $T_{\frac{1}{2}}$이 현저히 짧아지는 rapid phase을 나타내고 1日當 50 ml 以上 사혈군에서는 짧아지는 程度가 완만한 slow phase을 나타낸다(Fig. 6).

2. 1日量 10 ml 및 25 ml 式 사혈한 群의 赤血球壽命을 測定하는데 있어 赤血球를 사용하였을 때에는 $T_{\frac{1}{2}}$ 측 정치에 유의한 差가 없었으며 이 범위 내에서는 Hct., Hb. 및 血淸鐵値도 역시 유의한 差가 없었다.

3. 1日量 50 ml 및 75 ml, 100 ml씩 사혈한 群에서는 赤血球만을 使用하였을 때와 全血을 試料로 하였을 때 사이에 $T_{\frac{1}{2}}$의 측정치에 유의한 差가 있었으며 이 때는 Hct., Hb. 및 血淸鐵値에도 變化가 있었다. 即, 全血을 사용한 赤血球 수명($T_{\frac{1}{2}}$)의 측정치가 赤血球만를 使用한 赤血球 수명($T_{\frac{1}{2}}$)의 측정치 보다 짧았다.

4. 一定기간(10日) 사혈의 총량(1000 ml)이 같을 때는 200 ml를 5回 사혈한 群이나 500 ml를 2

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回 사혈한 群 사이에 赤血球 수명($T_{\frac{1}{2}}$)에 有義한 差를 볼 수 없었다.

5. 직업적 공혈자의 반복사혈로 因한 慢性 빈혈환자 9名에서의 ^{51}Cr 赤血球수명($T_{\frac{1}{2}}$) 측정치는 平均 19.2日로 짧아져 있으나 赤血球壽命測定前後에 充分한 鐵劑를 投與하여 Hct., Hb. 및 血淸鐵値를 增加시켰으며 이때 볼 수 있었든 Hct値를 規準하여 교정한 赤血球수명($T_{\frac{1}{2}}$)은 거의 正常범위 안에 있어(27.6日) 이러한 因子를 고려하지 않으면 잘못 이해할 수가 있다.

6. 鉤虫仔虫을 구충한 7名의 中等度 鐵결핍성 빈혈환자에서의 赤血球수명($T_{\frac{1}{2}}$) 측정치는 25日~31日로 平均 28日이었으며, 이때 장 출혈량은 1日 1.0~3.5 ml이었다. 단시일내의 급성실혈시에는 이와같은 소량의 失血도 赤血球수명($T_{\frac{1}{2}}$) 측정치에 영향을 보여 줌을 알 수 있었다. 따라서 이러한 정도의 실혈은 실험오차에 기인하는 것인지 아니면 장기 출혈에서는 이러한 소량의 실혈이 赤血球수명($T_{\frac{1}{2}}$) 측정에 영향을 미치지 않는 것인지는 아직 확실히 말할 수 없다.

8. ^{51}Cr-標識赤血球로 측정한 赤血球수명($T_{\frac{1}{2}}$)은 측정시의 失血量에 큰 영향을 받음을 알 수 있으며 著者들은 ^{51}Cr-標識赤血球를 利用한 赤血球 수명 측정때 검사기간중 실혈량이 赤血球壽命値에 미치는 관계를 上述한 實驗値를 基礎로 하여 다음과 같을 校正式을 考案해 보았다.

$$^{51}Cr\ T_{\frac{1}{2}} = 17.0\,e^{-0.0495\times} + 18.4\,e^{-0.000924\times}$$

但 ×: 1日 失血量(單位 ml)

INTRODUCTION

Erythrocyte ^{51}Cr-tagging method is widely used in determination of red cell survival time. It gives, however, reliable results only when the red cell mass remains constant during the study.[1)]

Under such circumstances only, the variations of radioactive concentration in the red blood cell faithfully and solely reflect the rate of removal of the tagged red blood cell from the circulation.

In several pathological conditions such as increased hemolysis and/or decreased production of red blood cell it is not possible to measure the red cell survival using the traditional technique, because the red blood cell mass is subject to change during the study. In the event of unsteady state, it would be necessary to measure the variations in the total quantity of circulating ^{51}Cr red blood cell directly, but not the change in concentration. It is estimated that the survival time of ^{51}Cr red blood cell would be changed in the anemic subjects with blood loss resulting in the change of red blood cell mass.

However, little work has been done on the relationship between the blood loss and survival of red blood cell.

In this study, the authors have measured the ^{51}Cr red blood cell survival in chronic posthemorrhagic hypochromic anemic cases due to hookworm infestation, excessive blood drawn for transfusion, and during blood loss due to daily phlebotomy.

MATERIALS AND METHODS

Fifty six patients were included in the present study.

In twenty-six healthy medical students small amount of acute blood loss (experimental group A) was produced by daily phlebotomy in the amount of 10, 25, 50, 75, and 100 ml for 10 to 15 days. On the day before phlebotomy, ^{51}Cr tagged own erythrocytes were injected to each subject.

Fourteen healthy medical students, in which a total amount of 1,000 ml of blood has been drawn, were divided into 3 sub-group according to the phlebotomy schedule. Hundred milliliter of blood has been drawn daily for 10 days in the first subgroup, 200 ml. every other day in the second, and 500 ml. twice in 10 days in the third subgroup.

These students were subjected this study as a category of large amout of acute blood loss (experimental group B).

Nine chronic posthemorrhagic anemia subjects caused by repeated phlebotomies for transfusion (experimental group C) were studied, and seven dewormed subjects with moderate iron deficiency anemia who had been infested with A. duodenale and never used iron preparation (experimental group D) were included in this study.

All the subjects were free from the intestinal bleeding and occult blood in the stools.

Measurement of red cell survival

T 1/2 ^{51}Cr. The procedure of Gray and Sterling[2] modified by Read[3] for labeling erythrocytes, was used. To 20~30 ml of blood, 100 uCi $Na_2{}^{51}CrO_4$ were added and each experimental subject was injected intravenously with 8~10 ml of labeled blood, leaving 2ml of blood for preparation of standard.

Blood samples were taken at 15, 30 minutes, 24 hours after injection and daily for 14 days thereafter.

^{51}Cr results were expressed as both counts per unit volume of whole blood and red blood cells and they were not corrected for elution.

Measurement of intestinal blood loss

The method for measurement of blood is described in this journal.[4]

RESULTS

I. ^{51}Cr red blood cell half-life (T 1/2) and acute blood loss.

(a) Small amount of phlebotomy group (experimental group A)

Table 1 and Fig. 1, 2, 3, 4, and 5 summarize

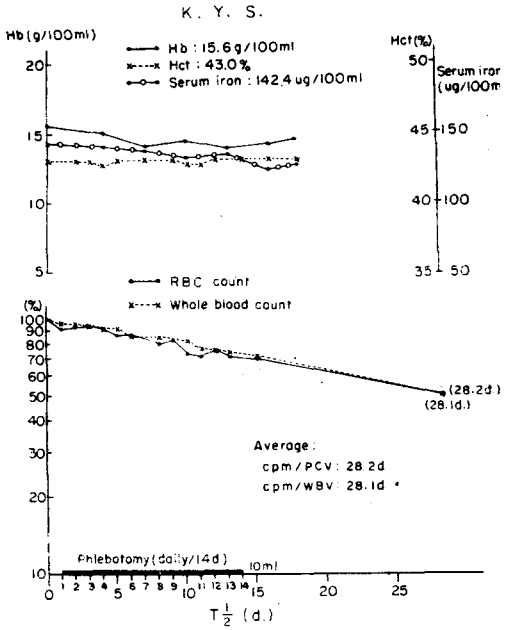

Fig. 1. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 10ml daily for 14 days.

Table 1. Average hematological values and ^{51}Cr red cell survival times in various experimental series

	Experimental series		No. cases	Hb (g/100 ml) Begin	Hb End	Hct (%) Begin	Hct End	Serum iron (μg/100 ml) Begin	Serum iron End	Red cell survival time (d) (expressed) cpm/whole blood	cpm/rbc mass
Acute blood	Small amount phlebotomy (Exp. group A) (daily/14d).	10 ml	6	15.1	14.9	45.3	44.7	132.7	103.5	28.1	28.2
		25 ml	6	14.6	14.1	44.5	42.0	131.6	112.2	22.6	22.8
		50 ml	4	14.3	12.6	41.5	36.0	109.9	68.4	15.4	17.4
		75 ml	4	13.0	12 2	41.	37.	97.9	63.6	13.3	17.2
		100 ml	6	14.3	13.0	43.5	38.8	104.4	64.4	13.1	16.2
	Large amount phlebotomy (Exp. group B)	100 ml×5	6	14.3	13.0	43.5	38.8	104.4	64.4	13.1	16.2
		200 ml×5	4	14.5	12.1	43.0	39.0	127.2	103.6	13.6	16.6
		500 ml×2	4	14.5	11.8	46.0	39.5	97.0	60.8	13.0	16.5
Chronic iron def. anemia	Post hemorrhagic anemia (Exp. group C)		9	6.4	11.9	24	35	47.0	77.2	19.2 (27.6)*	
	Hook-worm anemia (Exp. group. D)		7	9.5		35		59.4		28.0	

* Number in parenthesis is ^{51}Cr red cell half-life corrected with Hct values.

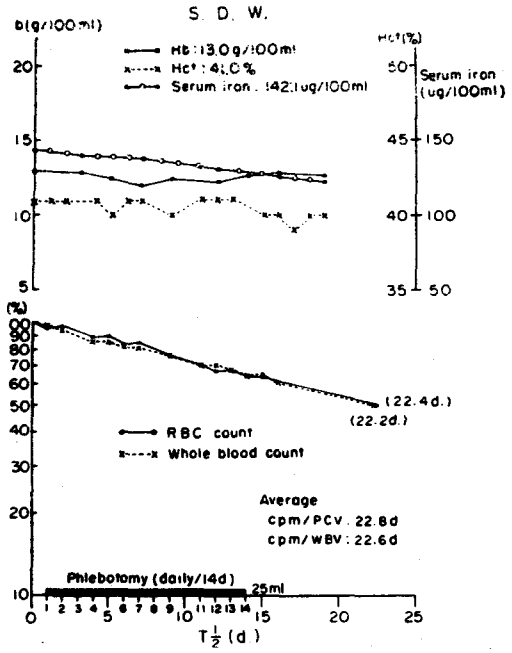

Fig. 2. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 25 ml daily for 14 days.

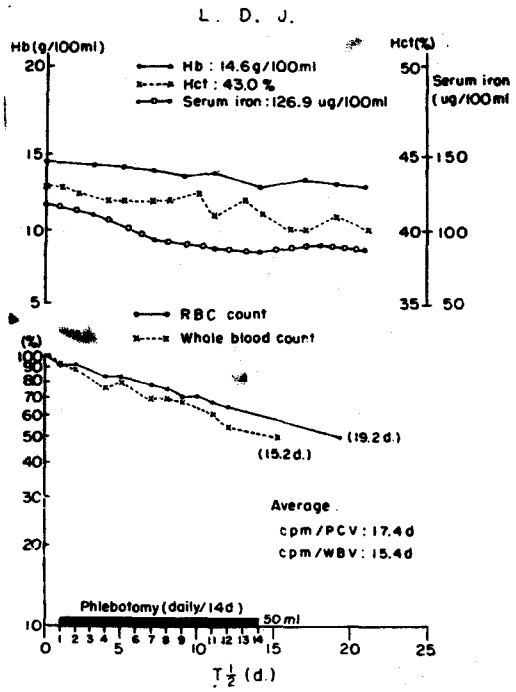

Fig. 3. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 50 ml daily for 14 days.

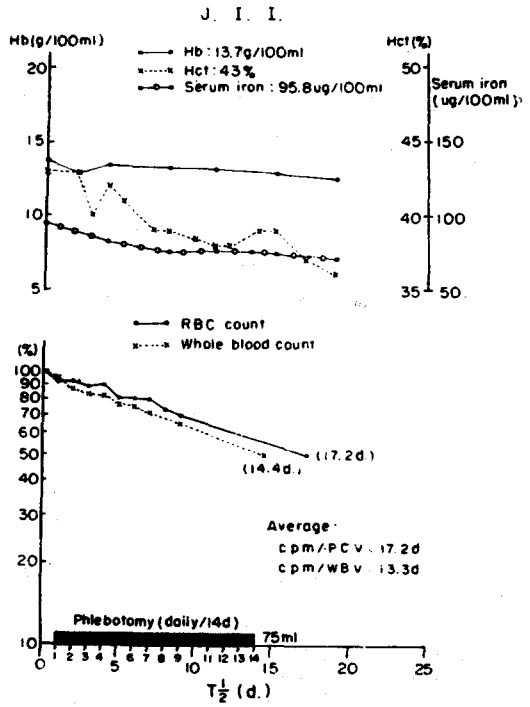

Fig. 4. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 75 ml daily for 14 days.

the results in the experimental group A. In the group of phlebotomy of 10 ml daily, ^{51}Cr red cell half-life measured by cpm/ml of red blood cell mass (RBCTm) was 28.2 days.

When the daily amount of phlebotomy increased to 25 ml, 50 ml, 75 ml, and 100 ml, the ^{51}Cr red blood cell half-life was shortened to 22.8, 17.4, 17.0 and 16.2 days, respectively. The more the blood loss, the shorter the ^{51}Cr red cell half life was.

The rate of shortening, however, was found to be considerably slow when the daily blood loss exceeded 50 ml. The ^{51}Cr red cell half-life measured by cpm/ml of whole blood (WBTm) in case of daily phlebotomy amounting 10 ml to 100 ml, as in above, were 28.1, 22.6, 15.4, 13.3 and 13.1 days, respectively (Tab. 1, Fig. 1, 2, 3, 4, 5, and 6).

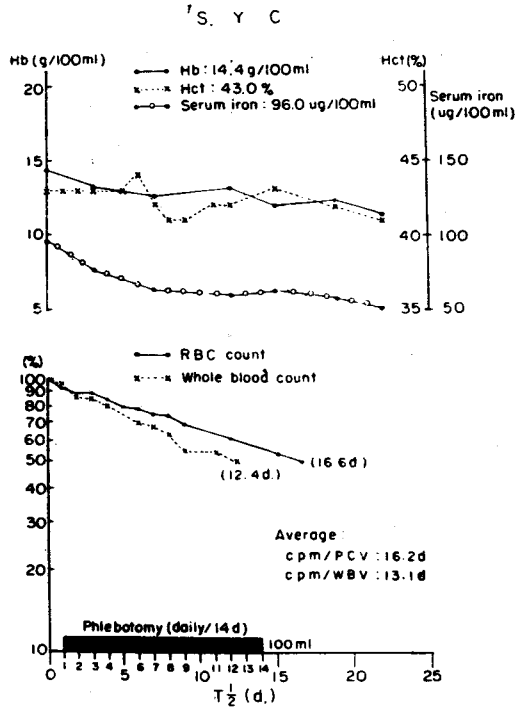

Fig. 5. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 100 ml daily for 14 days.

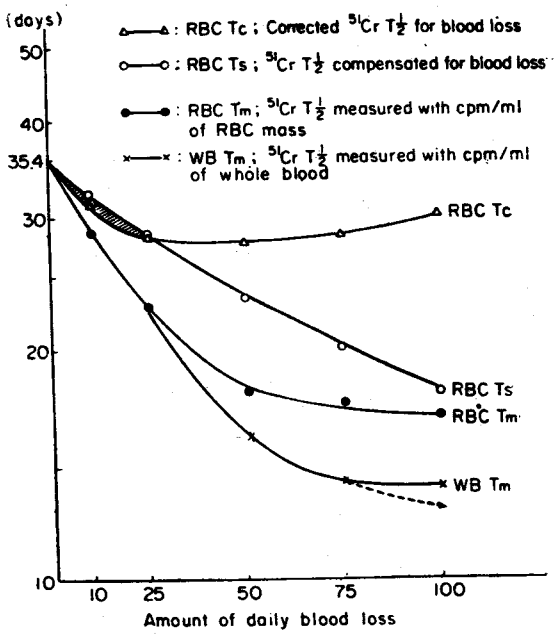

Fig. 6. Effect of acute blood loss on ^{51}Cr red cell half-life ($T\frac{1}{2}$) measured with various conditions.

As shown above, the ^{51}Cr red cell half-life following phlebotomy of 10 ml and 25 ml was almost same regardless of measurement by red cell mass or whole blood. The value, (WBCTm), however, was more shortened in daily phlebotomy exceeding 50 ml, than that measured by cpm/ml of red cell mass.

The values of hematocrit, hemoglobin and serum iron were also decreased. The difference between the values RBCTm and WBTm is believed to be due to decrease in red blood cells and hematocrit.

When the values were corrected for ^{51}Cr lost due to blood loss (RBCTc) they should have been 31.1, 28.3, 27.5, 28.0 and 29.8 days, respectively, and lengthening of the values instead of shortening in daily phlebotomy exceeding 50 ml has been observed. This can be explained by the possibility that the production of red blood cell can not reach the amount of blood loss and it gives a faulty impression of lengthening. If the amount of blood loss is completely compensated theoretically, the values of ^{51}Cr (RBCTs) should be 32.0, 28.2, 23.4, 20.0, and 17.5 days, respectively.

Actual lenghthening of ^{51}Cr red cell half-life corrected for ^{51}Cr loss(RBCTc) is believed to be due to lowered dilution effect of total red cells by less production of red cells (Fig. 6.)

b) Large amount of phlebotomy group (experimental group B)

Results in experimental group B were illustrated in Table 1 and Fig. 5, 7 and 8.

There was no significant difference among the three subgroups in ^{51}Cr half-life measured with both cpm/ml of whole blood and red cells.

However, there was a tendency of shortening of ^{51}Cr red cell half-life, although it is not as

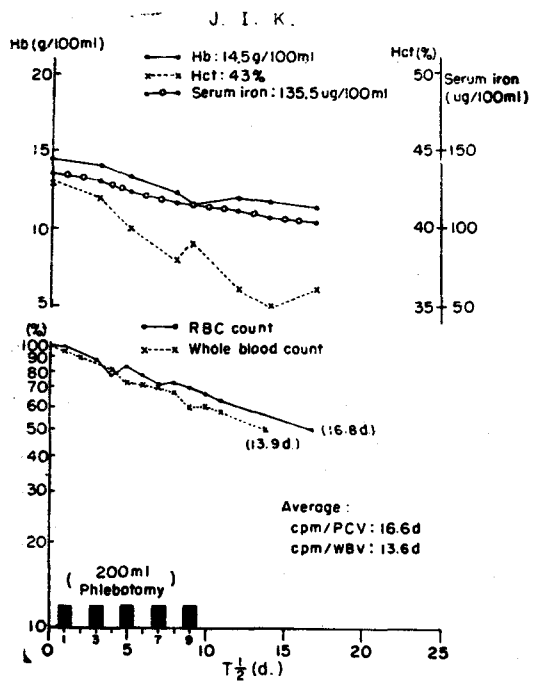

Fig. 7. Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 200 ml every other day for 10 days.

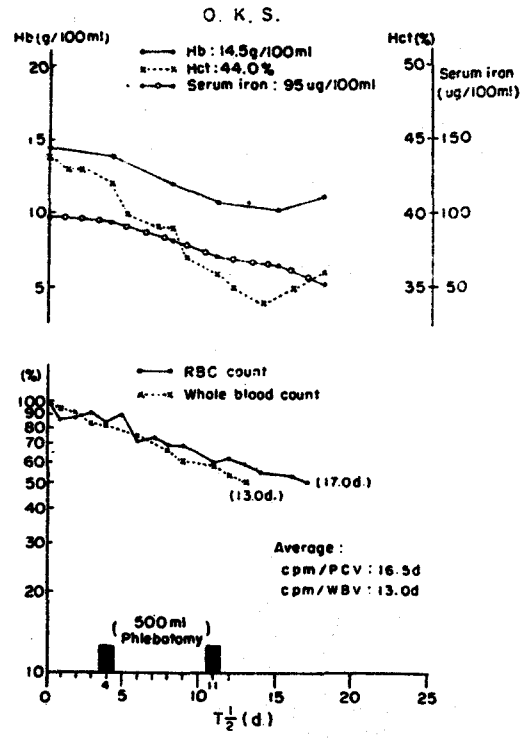

Fig. 8 Hematological data and ^{51}Cr red cell half-life during blood-lettings in the amount of 500 ml twice for 10 days.

Table 2. Red cell life spans and laboratory findings in chronic posthemorrhagic subjects (professional blood donors)

Cases	Hct (%) Begin of survival	Hct (%) End of study	Hct increase during study (%)	Hb (g/100 ml) Begin of survival	Hb (g/100 ml) End of study	Hb increase during study (g/100 ml)	Serum iron (ug/100 ml) Begin of survival	Serum iron (ug/100 ml) End of study	Serum iron increase during study (μg/100 ml)	Measured $T\frac{1}{2}$ ^{51}Cr (d.)	Corrected $T\frac{1}{2}$ ^{51}Cr (d.)	Treatment
1	24	35	11	6.8	12.1	5.3	48.8	80.5	31.7	18.5	26.9	oral iron
2	29	37	8	7.8	12.2	4.4	60.0	82.5	22.5	2e.9	27.9	oral iron
3	27	36	8	7.4	12.1	4.7	65.6	75.0	9.4	20.2	25.9	oral iron
4	25	34	9	6.6	12.0	5.4	42.4	76.5	34.1	22.1	30.0	oral iron i.m. iron
5	25	35	10	6.6	11.8	5.2	49.6	78.5	28.9	22.1	30.9	oral iron i. v. iron
6	19	35	16	5.3	12.2	6.9	38.5	75.0	36.5	15.2	28	oral iron i.v. iron
7	31	34	3	7.0	11.7	4.7	52.0	85.5	33.5	24.6	26.9	oral iron
8	18	33	15	5.5	11.5	6.0	35.6	70.8	35.2	14.2	26.0	oral iron i. m. iron
9	18	35	17	5.0	11.8	6.8	30.8	70.5	39.7	13.9	26.4	oral iron i.v. iron
Average	24	35	9	6.4	11.9	5.5	47.0	77.2	30.2	19.2	27.6	

Table 3. Red cell life spans and laboratory findings in hookworm anemia subjects after deworming

Cases	Ery. (mill)	Hb (g/100 ml)	Hct (%)	Serum iron (μg/100 ml)	MCHC (%)	$T\frac{1}{2}$ ^{51}Cr (d.)	Blood loss (ml/d.)
11	2.86	8.5	30	45.5	28.3	26	(—)
12	3.42	9.5	36	56.5	26.3	28	3.5
13	3.57	9.0	34	50.8	27.9	29	1.7
14	3.67	10.0	40	68.2	25.0	26	3.1
15	3.80	9.8	38	68.7	25.8	25	(—)
16	2.96	9.7	33	60.4	29.4	28	3.3
17	3.55	10.0	35	66.9	28.5	31	(—)
Average	3.40	9.5	35	59.4	27.3	28	

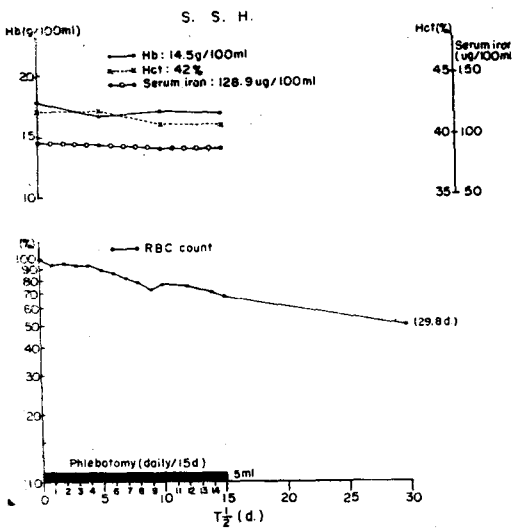

Fig. 9. Hematological data and Cr red cell half-life during blood-lettings in the amount of 5 ml daily for 15 days.

significant as in the small amount of phlebotomy (experimenal group A).

II) ^{51}Cr red blood cell half-life (T 1/2) and chronic posthemorrhagic anemia

a) Chronic iron deficiency anemia (experimental group C)

All the subjects in this experimental group(C) presented a history of 10 to 12 blood drawings of 380 to 480 ml every year over a peroid of the past 5 years.

Phlebotomy was withheld and iron preparation was given for at least one month prior to this study, resulting in the increase of hemoglobin of 5.5 g/dl and of hematocrit of 9%. The measured ^{51}Cr red cell half-life was shortened with a range from 13.9 to 22.1 days, the average being 19.2 days. In a non-steady state, the measurement of radioactivity/ml of red cells is not reliable, while in a steady state where the red cell mass is constant, the measurement of radioactivity/ml of red cells is more reliable. In a non-steady state, one can obtain better estimates by measuring the specific activity of whole blood.[1]

However, Strumia[5] has reported that corrections of the values obtained must be made according to the variations in the volume of red cells caused by the rate of new red cell production. In this series, ^{51}Cr red cell half-life was corrected for rate of change in hematocrit value between the end and the start of ^{51}Cr study,[6] and it was almost similar to data(RBCTc) obtained in acute blood loss group. It was found that the corrected ^{51}Cr red cell half-life was lengthened to normal range (Table 1 & 2).

b) Hookworm anemia(experimental group D)

Seven dewormed subjects with moderate iron deficiency anemia who had been infested with A. duodenale and never used iron preparation were included in this study and Table 1. and 3 illustrate the hematological data and ^{51}Cr red cell half-life.

The hematocrit values ranged from 30 to 40%, the average being 35% and their hematological values were almost invariable during the study.

^{51}Cr red cell half-life ranged from 25 to 31 days, the average being 28 days which is within the normal range of variation although hypochromic anemia and small amount of intestinal blood in the feces(1.0～3.5ml/d) were found in some cases.

Blood loss of such an amount is not of sufficient magnitude for the observed decrease in erythrocytes survival time. However, judging from our experimental data, ^{51}Cr red cell half-life hase been shortened even by daily loss of 3 ml or so (Fig. 9). This extent of shortening might also be happened by the normal ranging of experimental error.

DISCUSSION

Literatures on red cell survival in posthemorrhagic (iron deficiency) anemia caused mainly by blood loss are scarce[7,9] and they are still contradictory. The span of iron deficient red cells in circulation is assumed to be normal by many investigators.[9,10,11,12,13,14,15]

Rash et al,[16] Verloop et al.,[17] Layrisse et al.,[18] Alvar Loria et al.,[19] Hamilton et al.,[9] Shirakawa,[20] and Keiderling et al.[21] found that life span is shortened in iron deficiency anemia.

Layrisse et al.[18] demonstrated in hookworm-infested subjects that excess hemolysis takes place in severe iron deficiency anemia, with marked changes in morphology of the red cells in addition to intestinal blood loss, and they represent evidence suggesting that the spleen plays an important role.

Hamilton and Sheets[9] have raised the possibility that the abnormal survival might be due to a toxic effect unrelated to lack of iron. Farid et al.[22] demonstrated a close relationship between the severity of iron deficiency anemia and the shortened red cell half-lif, and the half-life was found to be shortened in all patients with hemoglobin value under 6.5 gm per 100 ml, regardless of intestinal blood loss.

Layrisse et al.,[18] however, demonstrated that there was a poor correlation between ^{51}Cr red cell survival, and the amount of blood loss. Temperley et al.[12] reported the blood loss of 3～17 ml per day in feces would not alter the ^{51}Cr survival in the blood to any significant degree.

Information on red cell survival in experimentally phlebotomized anemic subjects and animals is also scarce.

Bale[23] and Bush[24] found normal red cell survival in experimentally phlebotomized anemia animals. On the other hand, Neuberger and Niven[25] Berlin and Lotz,[26] Fedorov,[27] and Shirakawa[20] reported the shortened red cell life span and they have suggested that red cells produced in response to acute hemorrhage are functionally poor from the stand point of life span, although adequate in numbers to replace rapidly the red cells lost.

It seems likely that red cell half life in iron deficiency anemia is maintained normally as long as a steady state without bleedings continues during the ^{51}Cr study.

In summary of literatures reviewed, the erythrocytes survival studies in iron deficiency anemia due to blood loss have never been satisfactorily concluded.

The following possibilities could be put under consideration for such controversies.

First, most subjects under study were hemorrhagic anemia patients caused by blood loss due to hookworm infestation and no detailed information on the extent of anemia or amount of blood loss was given. Production rate of erythrocyte in bone marrow in those subjects during observation was also not estimated.

Second, most of reports gave no quantitative measurement of blood loss which was the main

iuse of iron deficiency anemia. Even when ieasured, they were not corrected to observed ^{51}Cr curves.

Third, consideration should be taken into account whether or not they were treated with iron uring the ^{51}Cr study.

Fourth, ^{51}Cr elution rate during the study hould be considered.

As described above, determination of ^{51}Cr red ell half life in unsteady state is not simple and nany influencing factors should be under consideration. Correction of amount of blood loss nay be the first requirement.

The methods for correction have been reported, but they were obviously inaccurate and oversimplified in view of the complexity of the problem ınd they are not satisfactory.

In normal values so far reported, there were no regards on exact amount of blood samplings which might cause blood loss.

Is not considering the exact amount of blood sampling one of the factors causing some differences in normal erythrocyte survival time by various reporters?

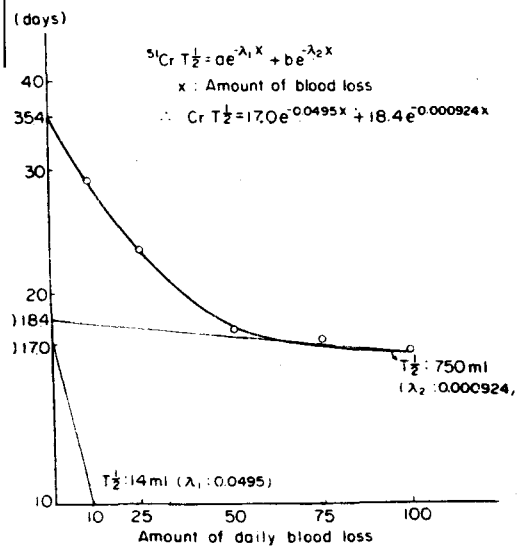

Fig. 10. Effect of acute blood loss on ^{51}Cr red cell half-life ($T\frac{1}{2}$).

Based on our experimental data, red cell half-life would be approximately 25 days when the daily amount of blood sampling reaches 20 ml for 2 weeks (Fig. 6).

The authors have postulated a formula which expresses ^{51}Cr red cell half-life measured in the subjects with blood loss during ^{51}Cr study (Fig. 10).

$$^{51}Cr\ T\ \tfrac{1}{2} = ae^{-\lambda_1 X} + be^{-\lambda_2 X}$$

Where X; amount of daily blood loss

a ; initial compartment

b ; second compartment

λ_1; decay constant of intial compartment

λ_2; decay constant of second compartment

"a" indicates 17.0 and "b". 18.4days as shown in our experimental data (Fig. 10). Then

$$^{51}Cr\ T\ \tfrac{1}{2} = 17.0\,e^{-0.0495\times} + 18.4e^{-0.000924\times}$$

This formula can be applied only for acute blood loss during ^{51}Cr study and further detailed experimental study should be followed in chronic posthemorrhagic anemia.

CONCLUSION

The ^{51}Cr method for measurement of red cell survival is applicable only in the steady state of equation between red cell production and destruction and stable red cell mass.

When there is a change in red cell mass due to blood loss, hemolysis, suppression of red cell production or ineffective erythropoiesis, the result is often misled.

In this paper effect of blood loss on ^{51}Cr red cell survival during the study is presented.

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