Reprint #4

RADIOIRON TRACER STUDIES: CLINICAL APPLICATIONS IN HEMATOLOGY

Scientific Exhibit

DNA1.940928.099

American Medical Association

by

R. K. Loeffler, M.D., V. P. Collins, M.D.,

C. T. Teng, M.D., and W. D. West, M.Sc.

Baylor University College of Medicine

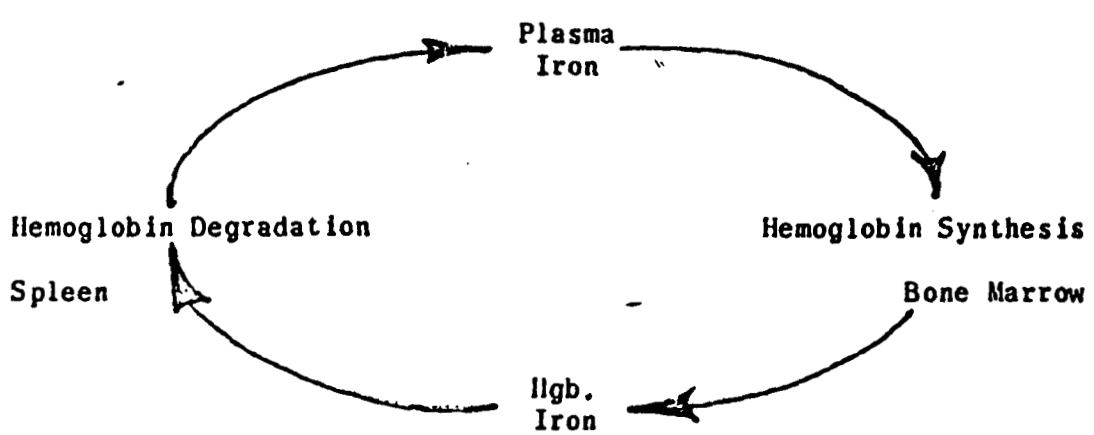
An adult man has 3.5 - 4.5 gm. of iron in his body. From 60 to 70% of this iron is in the red cells as hemoglobin iron, less than 1% in the circulating plasma as plasma iron attached to a protein, Transferrin; and the rest is in other forms of body irons (storage, myoglobin, resp. enzymes).

Although the plasma iron represents less than 1% of the total body stores, it is conveniently available for uniform sampling. It is in labile equilibrium with iron involved in the hemoglobin cycle and in other forms throughout the body.

The hemoglobin cycle consists of three main steps:

1. Synthesis of hemoglobin from iron available in bone marrow.
2. Degradation to available Fe of hemoglobin liberated by breakdown of aged or defective red cells in spleen or other sites.
3. Transport of iron as plasma iron from sites of breakdown and storage to bone Marrow.

The Hemoglobin Cycle



The average life span of normal red cells is generally accepted as 120 days. This means that 100% of red cells are replaced every 120 days, or 0.83% are destroyed and replaced daily.

A 70 kg. man has:

(1) a blood volume of 5000 cc., with

(2) 5×10^6 red cells per mm^3

(3) 12 gm. of Hgb. per 100 cc. of blood, and

(4) 3.34 mg. of iron per gm. of Hgb.

The orders of magnitude for the various components in this hemoglobin cycle may be estimated in the following terms:

Number of red cells destroyed and replaced = 2.1×10^{11} red cells per day
or
2.4×10^6 red cells per second

Calculation:

(0.83×10^{-3})	x	$(5 \times 10^6 \times 10^3)$	x	(5×10^3)
Fraction of red cells destroyed or replaced		No. of red cells per cc. of blood		Total blood volume

<u>Amount of hemoglobin degraded and synthesized</u> = 6.2 gm. of Hgb. per day

Calculation:

(0.83×10^{-2})	x	(15×10^{-2})	x	(5×10^3)
Fraction of hemoglobin degraded and synthesized		Gm. of hemoglobin per 100 cc. of blood		Total blood volume

<u>Amount of iron required for (1) and (2)</u> = 21 mg. of Fe per day

Calculation:

6.2	x	3.34
Gm. of hemoglobin synthesized per day		Mg. of Fe per gm. of hemoglobin

Since 21 mgm. of iron is the daily requirement for hemoglobin production, and since the total iron in circulating plasma is relatively constant at approximately 3 mg., then iron must enter the plasma (from the spleen and other sources) at the same rate at which it leaves (to be incorporated in hemoglobin in newly formed red cells or to enter storage depots).

As mentioned above, the plasma iron pool is in equilibrium with all compartments of body iron, the rate of iron leaving the plasma must therefore represent a composite rate of all pathways involved. However, since the rate of iron leaving for storage is comparatively slow, that for excretion and other purposes even slower, it may be assumed, with few exceptions, that the rate of iron leaving the plasma is by and large a reflection of the functional status of hemopoiesis. Exceptions are pernicious anemia and Mediterranean anemia.

A more direct and more accurate index of hemopoietic activity is the rate of incorporation of iron in the new formed red cells.

Under most hematological conditions, the rate of plasma iron disappearance and the rate of incorporation of iron in red cells are directly related. One exception is seen in untreated pernicious anemia where a rapid plasma iron disappearance is associated with sluggish iron uptake, the latter is presumably evidence of the so-called maturation arrest.

It is obvious that the determination of these rates adds the dimension of time and brings the concept of dynamics to the understanding of the physiology of iron and hemoglobin metabolism in health and disease.

Both of these rates can be measured by the use of Fe^{59} as a tracer. It is the purpose of the exhibit to demonstrate the usefulness of tracer iron turnover study in the diagnosis and prognosis of various hematologic conditions.

The equipment necessary is a well-type scintillation counter and scaler. This can be of a dual purpose design so that the same facilities can be used for the determination of radioiodine uptake in thyroid studies. It is predicted that the radioiron turnover test will become as useful in the study of anemia as radioiodine in the study of thyroid disease.

Normal and Abnormal Values Encountered
with Radioiron Tracer Studies

Hematological Disorders	$(Fe)_p$	Fe^{59} T.O. Half Time	Uptake of Fe^{59} by RBC
	ug%	Hour	%
Normal	70-120	1-2	Up to 90% in 1 wk. Then plateau
Anemias			
Iron deficiency	0-50	0.3-0.5	Approaching 100% in 3-4 days Then plateau
Chronic blood loss	0-50	0.3-0.5	Approaching 100% in 3-4 days Then steady fall off
Hemolytic	15-200	0.3-0.5	Approaching 100% in 3-4 days Then plateau
Chronic infections Malignant tumors Leukemias Liver diseases Uremia	40-60	0.5-1	
Aplastic and Myelophthisic	150-200	2-4	Very low and slowly rising
Anemias			
Addisonian untreated	150-200	0.3-0.5	Slow rise over many weeks, no plateau
remission			
early	40-70	0.3-1	approaching normal
late	70-120		
Mediterranean	150-200	0.3-0.5	Slow rise over many weeks, no plateau
Polycythemia Vera			
untreated	150-200	0.3-0.5	approaching 100%
remission	70-120	1-2	normal
repeated phlebotomy	40-70	0.3-0.5	approaching 100%
Hemochromatosis	200-250		

TECHNIQUE

1. Withdraw 10 cc. of blood into a heparinized syringe for whole blood and plasma samples (1.0 - 2.0 cc.) for the determination of natural radioactivity.

2. Inject intravenously 5.0 - 10.0 cc. of Fe^{59} citrate solution containing 4 microcuries of Fe^{59}. Avoid venostasis.

3. Obtain heparinized blood samples (5 - 10 cc.) at 5, 15, 30, 60, and 120 minutes after injection. Avoid venostasis. Pipette 1.0 - 2.0 cc. aliquot of whole blood from the 15 minute sample, and 1.0 - 2.0 cc. of plasma from from all samples into counting tubes.

4. Count all samples from steps 1 and 3.

5. Plot plasma activity (log scale) against time (linear scale) on semi-log paper.

6. Extrapolate to obtain zero time plasma activity. Determine disappearance or turnover half time ($T\frac{1}{2}$) of plasma Fe^{59}.

7. Calculate plasma volume: $PV = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. plasma}}$

8. Calculate hematocrit from activity of 15 minute samples:
$Hct = 1 - \text{Plasmacrit} = 1 - \frac{\text{Counts/cc. whole blood}}{\text{Counts/cc. Plasma}}$

9. Calculate zero time whole blood activity by proportionality:
Zero time counts/cc. Whole Blood = Zero time counts/cc. Plasma x Pct.

10. Calculate blood volume:
$B.V. = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. whole blood}}$

11. Pool plasma samples, determine iron concentration on duplicate aliquots.

12. Obtain blood samples, (5 cc., heparinized), at 3, 7, and 14 days. Count 1.0 - 2.0 cc. aliquot.

13. Determine approximate per cent red cell incorporation of Fe^{59}:
$\% \text{ uptake} = \frac{\text{Count/cc. follow-up blood sample x 100}}{\text{Zero time count/cc. whole blood}}$