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EXISTENCE OF AN ERYTHROPOIETIC LABILE IRON POOL

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During recent years the use of radioiron (Fe 59) as a tracer has elucidated some fundamental aspects of the physiology and biochemistry of iron kinetics. Pollycove and Mortimer (1961) have proposed mathematical models to explain the major pathways of iron kinetics in man 1 (Fig. 1). The concept of a labile erythropoietic iron pool 1 is of central importance to these models. So far, no physiological or biochemical entity corresponding to such a labile iron pool has been demonstrated. In the present series of experiments, an attempt was made to study some components of iron kinetics in the dog and rabbit, with a view to demonstrate the existence of an erythropoietic labile iron pool. Graded doses of 2.5 percent aqueous solution of phenylhydrazine were injected subcutaneously in rabbits to produce reticulocytosis. For each experiment 30 to 100 microcuries of radioiron (supplied by Abbot Laboratory, Oak Ridge), as ferrous citrate specific activity 3-12 c/ug, was incubated at 37° C for 1/2 hour with sufficient quantity of fresh plasma from dog/rabbit, to ensure binding of all iron to the Beta 1-globulin transferrin. After incubation, a measured amount of Fe 59 labelled plasma was injected intravenously in the dog/rabbit and blood samples were collected from the dog/rabbit at different time intervals depending upon the nature of the experiments. The experimental animals were then killed and samples of blood, bone marrow, ribs, rib-marrow, and pieces of liver and spleen collected for biochemical studies. Radioactivity in the different biological samples was measured by counting 1-2 ml aliquots in a scintillation well counter. Complete iron kinetics studies were performed in two dogs for a period of ten day as described by Pollycove (2) and Pollycove and Mortimer (1). By

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applying the mathematical model to radioiron measurements of plasma, red cells, liver, spleen, and sacral marrow, the intercompartmental rate constant was calculated for trasfer of iron from the erythropoietic labile iron pool to erythron iron irreversibly fixed for heme synthesis (Fig. 2).

The data on 8 experimental dogs (dogs 1,2,5-10) showed that marrow radioiron was associated with cellular elements. Doubly crystallized heme was prepared from marrow aspirations of femur, marrow scrapings from ribs and intact rib homogenates of dogs after heme synthesis was arrested with 5×10^{-4} M lead acetate. Analysis of data showed that soon after injection of Fe 59 labeled plasma, marrow radioiron occurs chiefly in non-heme form, 8 hours after injection (6 hours after most of the radioiron initially in the plasma had accumulated in marrow) approximately half of the marrow radioiron was present as erythron non-heme radioiron (Fig. 3). Subsequently, increasing amounts of erythron radioiron occurred in heme as non-heme radioiron decreased. The *calculated* rates of transfer of radioiron from labile non-heme iron to fixed heme iron in dogs 3 and 4 (Fig. 2) are in good agreement with the direct marrow analyses in the other 8 dogs (Fig. 3). The non-heme radioiron was largely associated with red cell membrane, while heme radioiron was present almost entirely in the interior of the red cell (Table 1). In the case of phenylhydrazine treated rabbits with 90 percent reticulocytes, Fe 59 labeled reticulocytes were obtained by venesection 30 minutes after the intravenous injection of a tracer amount of transferrin bound radioiron. Subsequent incubation of these washed (3 X with unlabeled plasma) labeled reticulocytes with unlabeled rabbit plasma and acid citrate dextrose solution demonstrated considerable transfer of reticulocyte radioiron back to plasma and significantly less to the acid citrate dextrose solution (Table II). The data on rabbit erythrocytes and canine marrow indicate that a labile erythropoietic iron pool exists on the membranes of immature erythrons, and that this iron is derived from plasma transferrin bound iron and transfers back to plasma, as well as transferring irreversibly into erythrons for heme synthesis. Further work on man is being continued.

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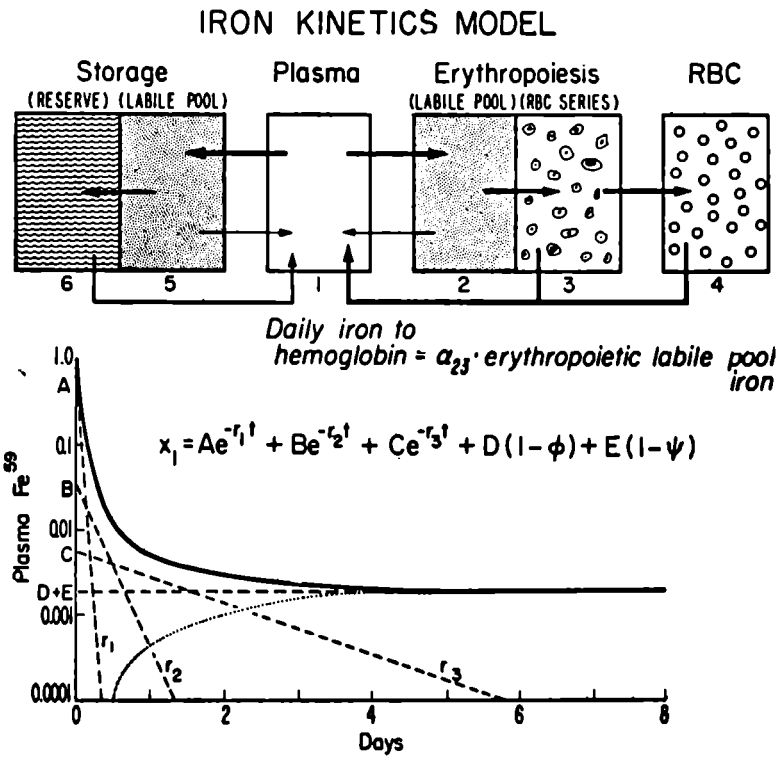

Fig. 1. General iron kinetics model. The intercompartmental rate constants from compartment i to compartment j, alfa ij are calculated from the measured slopes (gamma 1, 2, 3), intercepts (A, B, C), and fraction of radioiron irreversibly fixed in maturing erythrons. The amounts of iron in the labile pools are calculated from these intercompartmental rate constants and direct measurement of total plasma iron. Iron for hemoglobin synthesis is the product of the **fraction** of erythropoietic labile iron pool irreversibly fixed in maturing erythrons, alfa 23, and the **amount** of iron in the erythropoietic labile iron pool. The intercompartmental rate constant alfa 23 determines the rate at which iron in the erythropoietic labile pool is converted into heme iron.

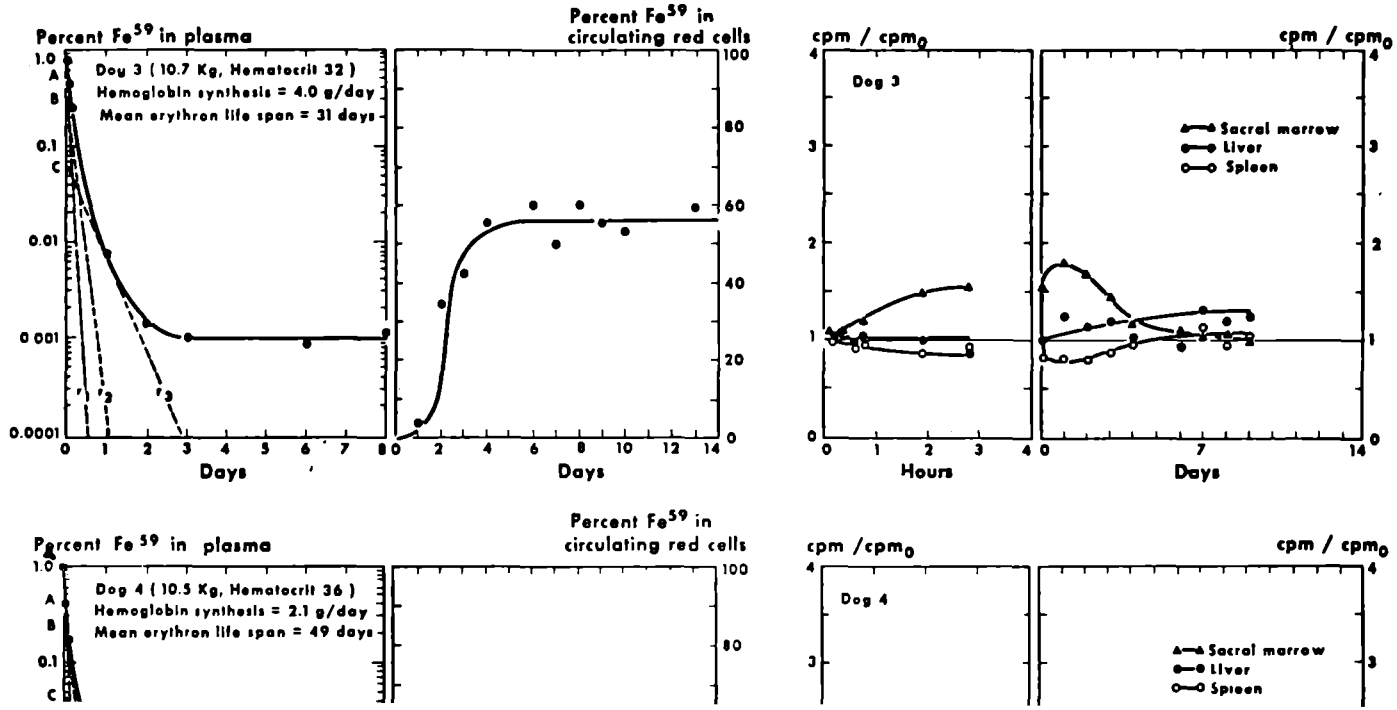
Percent Fe59 in plasma
Percent Fe59 in circulating red cells
Dog 3 (10.7 Kg, Hematocrit 32)
Hemoglobin synthesis = 4.0 g/day
Mean erythron life span = 31 days
Days
cpm / cpm0
Dog 3
Sacral marrow
Liver
Spleen
Hours
Days
Dog 4 (10.5 Kg, Hematocrit 36)
Hemoglobin synthesis = 2.1 g/day
Mean erythron life span = 49 days
Dog 4

Fig. 2. Iron kinetics data of dogs 3 and 4. Intercompartmental rate constant alfa 23 is calculated from measurements of the slopes gamma 1, 2, 3, their intercepts A, B, C, and the fraction of radioiron fixed in maturing erythrons, f.

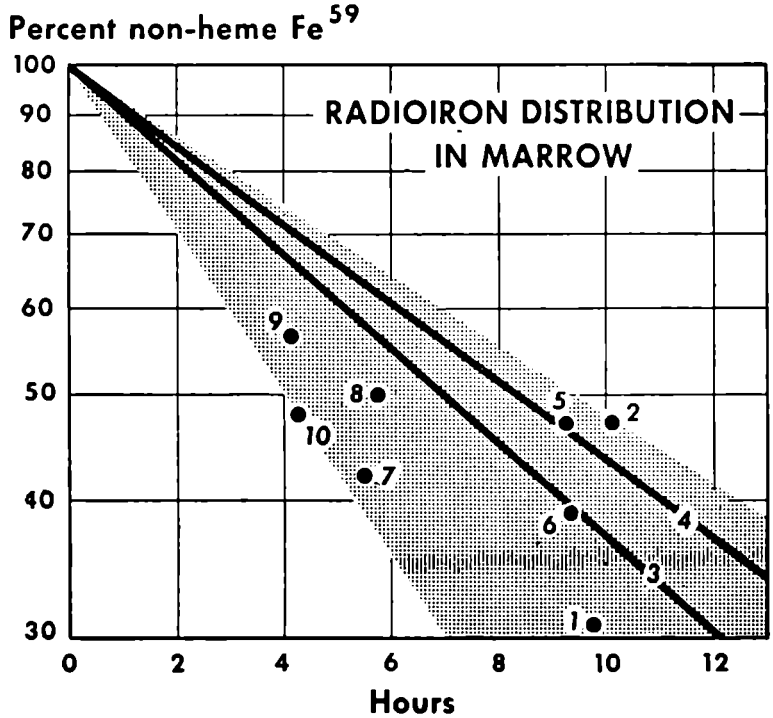

Fig. 3. Radioiron distribution in marrow. Points for dogs 1, 2, 5-10 were obtained from direct analysis of red marrow including measurement of Fe 59 specific activity of doubly crystallized heme. The slopes for dogs 3 and 4 are the calculated intercompartmental transfer rates from the erythropoietic labile iron pool to irreversibly fixed erythron iron, alfa 23. These rate constants were calculated by analysis in accordance with the mathematical model (Fig. 1) of plasma radioiron data shown in Figure 2.

RADIOIRON DISTRIBUTION IN MARROW CELLS

Dog	Percent non-heme Fe^{59}	
	Cell membrane	Cell interior
6	61	21
7	80	—
9	59	9
10	68	22

Table I. Distribution of radioiron between non-heme and heme in cell membrane and cell interior. Data corresponds to times after injection and distribution of radioiron in intact cells of the dogs shown in Figure 3.

RETICULOCYTE RADIOIRON TRANSFERRED TO INCUBATE

Incubate	Total iron binding capacity (gamma g/100 ml)	Percent reticulocyte Fe^{59} transferred
Phenylhydrazine plasma	418	3.9
Post-venesection plasma	329	3.2
Normal plasma	186	2.6
Acide-citrate-dextrose solution	0	1.7

Table II. Transfer of reticulocyte radioiron to plasma or ACD after one hour incubation at 37° C. Reticulocytes were initially labeled by **in vivo** transfer of Fe 59 from plasma transferrin.