

LIQUID SCINTILLATION COUNTING  
RECENT APPLICATIONS AND DEVELOPMENT  
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LIQUID SCINTILLATION COUNTING FOR  $^{59}\text{Fe}$   
AND  $^{51}\text{Cr}$  IN ERYTHROKINETIC STUDIES

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I. INTRODUCTION

The study of erythrokinetics in man requires the use of appropriate isotopes to evaluate red cell production and destruction. Red cell production is generally investigated through studies of iron kinetics and  $^{59}\text{Fe}$  is the chosen isotope. Red cell destruction is commonly evaluated through labelled red cell survival studies, and  $^{51}\text{Cr}$  is the most extensively employed label. These isotopes can be simultaneously.

In erythrokinetic studies, both  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  have been measured by solid scintillation counting, but results have often been unsatisfactory. In particular, the major difficulties were encountered in the investigation of iron kinetics. In these studies, after the injection of  $^{59}\text{Fe}$ -transferrin, it is necessary to measure the time course of  $^{59}\text{Fe}$  activity both in plasma and in whole blood. The assay of plasma  $^{59}\text{Fe}$  activity becomes critical after the first day of study, since, after 24 hours, less than 1% of the injected dose is still present in the plasma. On the other hand, in order to derive

useful information, the plasma  $^{59}\text{Fe}$  clearance curve must be followed until the 14th day.

In 1972, on the basis of previous traditional erythrokinetic studies (Perugini *et al.*, 1966; Storti and Perugini, 1969), we recognized that the first problem which had to be tackled was that of improving the accuracy of the experimental measurements.

The improvements of the techniques for liquid scintillation counting (LSC) led us to assume that the beta counting of  $^{59}\text{Fe}$  in iron kinetics might present some advantages over the conventional gamma counting. The excellent results obtained (Perugini *et al.*, 1974) led us later to develop a procedure for the simultaneous assay of  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  in whole blood by liquid scintillation counting (Cazzola *et al.*, 1976).

This paper gives a detailed account of the techniques developed in our laboratory and of the results obtained during the past years.

## II. $^{59}\text{Fe}$ ASSAY IN THE PLASMA

$^{59}\text{Fe}$  can be readily detected by gamma counting because of its energetic  $\gamma$ -rays (1.098 and 1.289 MeV). Photo-peak counting efficiency is about 15% (background 30-35 counts/min) with a 3-inch NaI (Tl) crystal.

It emits also beta particles with an average energy of 0.12 MeV. The assay of  $^{59}\text{Fe}$  by LSC has been employed mainly for research purposes, in order to study particular aspects of iron metabolism by means of the double tracer technique with  $^{59}\text{Fe}$  and  $^{55}\text{Fe}$ .

We decided to employ LSC for the determination of plasma radioiron activity in ferrokinetic studies with the aim of:  
(a) improving the measurement of the plasma clearance curve;  
(b) reducing the volume of plasma samples.

In our laboratory, the following procedures for LSC of  $^{59}\text{Fe}$  in the plasma was found to be optimal. 1 ml of plasma is placed in a glass LSC vial. 1 ml of a mixture of Soluene-350:isopropanol (1:2 v/v) is added, followed by a mixture of 15 ml Instagel:0.5N HCl (9:1 v/v). When plasma hemoglobin must be removed from plasma samples before counting, the procedure is modified as follows. Plasma hemoglobin is precipitated by the method of Cavill *et al.* (1976). 2 ml of supernatant is placed in a counting vial and mixed with 2 ml of the Soluene-350:isopropanol solution. Then, 14 ml of scintillator fluid are added.

Samples are counted in a Packard Tri-Carb 3320 liquid scintillation spectrometer operated at 10°C in a window of

50-1000 divisions with a gain of 7.0%. Quenching correction is made by means of an external standard ratio.

Using this procedure, counting efficiency ranged from 75 to 87% with a background of about 30 cpm. These values are in agreement with the results obtained with similar procedures by other authors (Fillet, 1971; Wagner, 1973). Using LSC of  $^{59}\text{Fe}$  we were able to improve consistently counting accuracy of the late points of the plasma  $^{59}\text{Fe}$  clearance curve. This allowed us to reveal variations in plasma radioactivity which cannot be easily seen by conventional gamma counting. Minimum detectable radioactivity (i.e. the activity which will give a count rate equal to twice the standard deviation on the background counting rate) is about ten times greater by LSC than by gamma counting. Further evidence for this is given by Fig. 1 which reports the clearance curves obtained by the two counting methods in one normal subject.

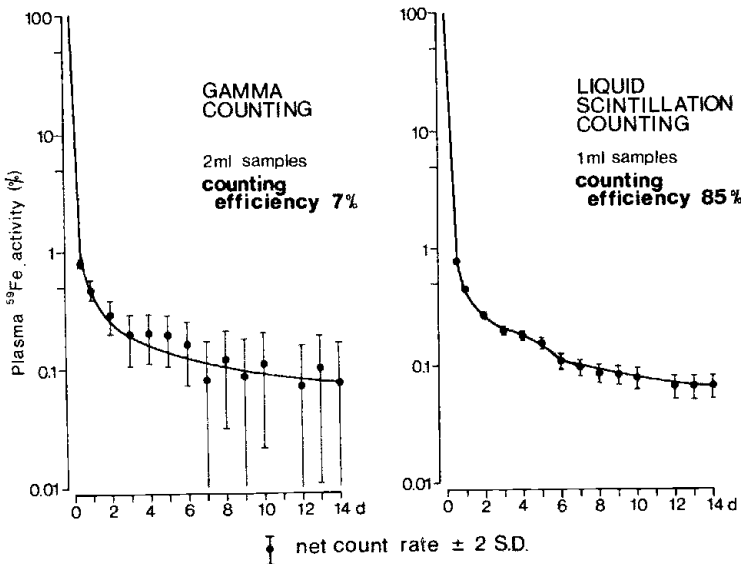


FIGURE 1. Plasma  $^{59}\text{Fe}$  clearance curves for a normal subject determined by gamma counting and by liquid scintillation counting: dots represent experimental data and full lines are model prediction. The 0.955 confidence limits of each net count rate are also indicated.

III. LSC OF  $^{59}\text{Fe}$  AND  $^{51}\text{Cr}$  IN WHOLE BLOOD

$^{59}\text{Fe}$  activity in red blood cells is very high after the first days of the ferrokinetic test, and gamma counting is a suitable method for measuring the red cell  $^{59}\text{Fe}$  utilization curve. If  $^{59}\text{Fe}$  is employed simultaneously with  $^{51}\text{Cr}$ , separate simultaneous measurements of the two isotopes in whole blood may be accomplished by solid scintillation counting of the photo-peaks since the gamma spectra of  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  are sufficiently different. However, counting efficiencies are low (about 15% for  $^{59}\text{Fe}$  and 2-3% for  $^{51}\text{Cr}$ ). For this reason, we decided to test LSC of the two isotopes.

$^{51}\text{Cr}$  decays by electron capture emitting 5 KeV X-rays and 4.5 KeV Auger electrons in 91% of the disintegrations, while the frequency of decay by gamma emission is only 9%. The Engberg plot of  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  in a 50-1000 division window (Fig. 2) demonstrated that the two isotopes are separable (Cazzola et al., 1976). We found that  $^{59}\text{Fe}$  may be counted with negligible  $^{51}\text{Cr}$  spillover in a channel of 200-800 divisions with a gain of 3.0%. The best compromise between  $^{51}\text{Cr}$  efficiency and  $^{59}\text{Fe}$  contribution was obtained in a channel of 50-500 divisions with a gain of 100%. Background was about 12 counts/min for  $^{59}\text{Fe}$  and 10 counts/min for  $^{51}\text{Cr}$  channel. Packed cell volume was the most critical quenching agent in whole blood.  $^{59}\text{Fe}$  efficiency ranged from 19.8% to 8.48%, while  $^{51}\text{Cr}$  efficiency ranged from 15.09% to 7.64% (packed cell volume from 0.15 to 0.54).

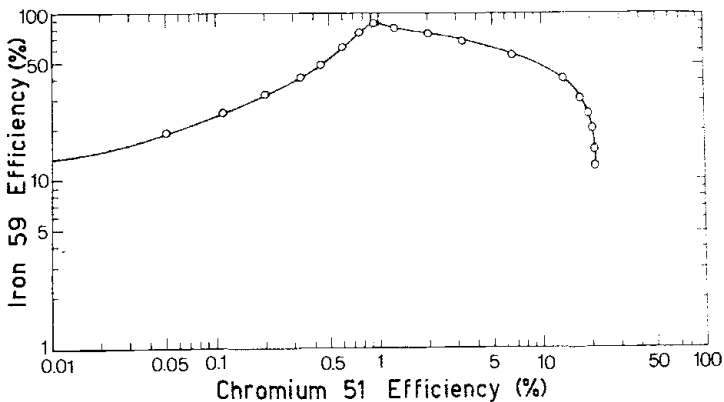


FIGURE 2. Engberg plot of  $^{59}\text{Fe}$  and  $^{51}\text{Cr}$  in a Packard Tri-Carb 3320 liquid scintillation counter. Window settings: 50-1000 divisions.

The following procedure was used for the preparation of counting samples. 0.2 ml of blood is pipetted into the counting vial and mixed with 1.5 ml of a Soluene-350:isopropanol solution (1:2 v/v). Then, 0.5 ml of 30%  $H_2O_2$  is added with immediate shaking. The vial is capped loosely and kept to room temperature for 60 min. Next, 15 ml of Insta-Gel:0.5N HCl mixture (9:1 v/v) is added and the vial is shaken vigorously.

In erythrokinetic studies,  $^{59}Fe$  and  $^{51}Cr$  could be simultaneously assayed by LSC with great accuracy. An illustrative example is shown in Fig. 3.

#### IV. CLINICAL APPLICATIONS

In ferrokinetic investigations, the plasma  $^{59}Fe$  clearance curve has been generally fitted by a sum of three negative exponential terms (Pollycove and Mortimer, 1961; Cook *et al.*,

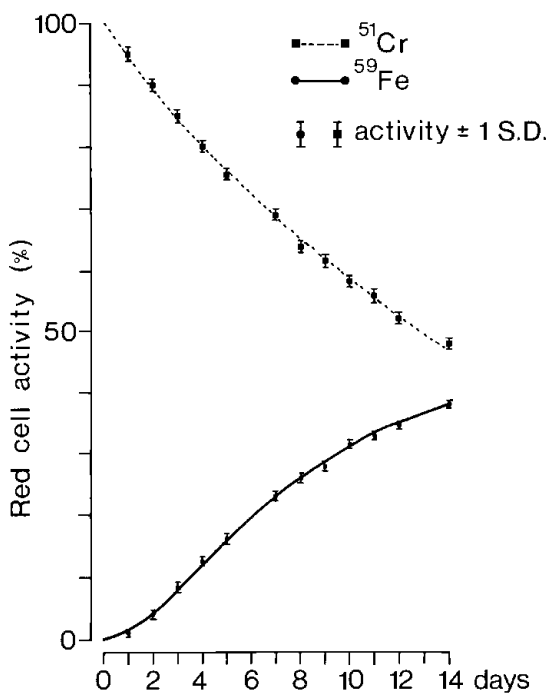


FIGURE 3. Red cell  $^{59}Fe$  utilization (—) and  $^{51}Cr$  labelled red cells disappearance (---) measured simultaneously by LSC in a patient with refractory anaemia. Net activity  $\pm 1$  S.D.

1970; Ricketts et al., 1975). We tested this approach and found it unsatisfactory for describing experimental curves obtained by LSC (Cazzola et al., 1979).

In normal subjects, the mean value for initial half-clearance time is about 90 minutes. After 2-3 hours the clearance rate becomes slower owing to radioiron returning to plasma from other compartments. In some normal subjects, we found a slight transient elevation in plasma activity or hump after 1-2 days. Such humps may be more evident in pathological conditions. In patients with marked peripheral haemolysis,  $^{59}\text{Fe}$  activity in the plasma may increase in the later part of the curve.

The transient elevations in plasma activity observed in normal subjects and in pathological conditions indicate a feedback of radioiron to the plasma from other compartments, i.e. from bone marrow because of ineffective erythropoiesis and from circulating red cells because of peripheral haemolysis. The recognition of these radioiron refluxes allowed us to describe iron kinetics by a seven-compartment model (Barosi, et al., 1978).

The accuracy of the ferrokinetic parameter estimates was found to be consistently better using data collected by LSC than data obtained by gamma counting (Barosi et al., 1976). This points out the importance of employing LSC in collecting the data whenever an interpretation by means of a mathematical model is the aim of the research.

A proper definition of radioiron reflux from red cells to the plasma proved to be essential for an accurate estimate of effective and ineffective erythropoiesis in severe haemolytic conditions (Stefanelli et al., 1979). Red cell death probability function is required for such definition. LSC of  $^{51}\text{Cr}$  and  $^{59}\text{Fe}$  allows one to obtain simultaneously accurate experimental data for estimating red cell death probability function and red cell iron turnover in these patients.

In conclusion, LSC is slightly more cumbersome than gamma counting as regards sample preparation and activity calculation. Nevertheless, it provides more accurate experimental data and permits a considerable reduction in the blood withdrawal and in the dose of isotopes to be administered. In our opinion, LSC has produced a remarkable advance in erythrokinetic studies.

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