

## Method of Separate Counting of the Blood Sample Labeled with $^{59}\text{Fe}$ and $\text{DF}^{32}\text{P}$ for the Determination of Iron Metabolism and Mean Red Cell Life Span

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$^{59}\text{Fe}$  and  $\text{DF}^{32}\text{P}$  are often used in series for the determination of iron metabolism and mean red cell life span. However, the radioactivity of these isotopes is counted more or less mixed each other in well-type scintillation counting for  $^{59}\text{Fe}$  and Geiger, or liquid scintillation counting for  $^{32}\text{P}$ . Therefore, it is necessary for the accurate determination of iron metabolism and mean red cell life span to count  $^{59}\text{Fe}$  and  $^{32}\text{P}$  of the blood sample separately.

To exclude the mixing of radioactivity of  $^{32}\text{P}$  in counting  $^{59}\text{Fe}$ , the methods of using channels ratio, decay ratio, and lead filter were investigated, and the use of lead filter was the simplest and most effective. Using 2 mm thick lead tube,

97% of radioactivity of  $^{32}\text{P}$  was cut at a sacrifice of 18% of radioactivity of  $^{59}\text{Fe}$ .

To exclude the mixing of radioactivity of  $^{59}\text{Fe}$  in counting  $^{32}\text{P}$ , use of aluminum filter, subtraction of  $^{59}\text{Fe}$  counts of the sample before injecting  $\text{DF}^{32}\text{P}$  from after, and Fe-heme extraction method were tested. Fe-heme extraction method was the best. By heme extraction with hydrochloric acid acetone, 98% of  $^{59}\text{Fe}$  was removed without any significant loss of  $^{32}\text{P}$ .

The method of heme extraction was applied for determining mean red cell life span of the blood sample with  $^{32}\text{P}$ , and the results were satisfactory.

## Determination of Red Cell Survival by Activation Analysis

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We used non-radioactive chromium ( $^{50}\text{Cr}$ ) to determine the red cell survival by neutron activation of the serial blood samples. This method will give the benefit to pregnant females and children whom the radioactive materials are not favored.

Fifty ml of patients blood was withdrawn and incubated with 0.5mg of  $^{50}\text{Cr}$  for 30 minutes at room temperature. Labeling process was ceased 10 minutes later by adding 100mg ascorbic acid

which reduces chromium ions of hexavalent to trivalent. After reinjection of labeled blood to host patients, 1ml samples of blood were withdrawn at intervals over a period of a month.

Samples were centrifuged, red cells were then freeze dried and transferred to quartz vials. Neutron irradiation was performed in the reactor of the Japan Atomic Energy Research Institute. The neutron flux was  $5 \times 10^{13}$  neutron/cm<sup>2</sup> sec and irradiation period was 21 hours. After the

neutron irradiation, samples were left in a container for about 2 weeks. No chemical separation, and even no opening of quartz vials is necessary before sample counting. A lithium-drifted germanium detector and 4096 channels pulse-height analyzer were used for gamma-ray spectrometry of neutron activated samples. The counts of the initial samples obtained 24 hours after the injection designated as 100% activity, and half-lives were considered as the dates where the curves of

graph cross 50% lines.

The studies were so far performed on 2 rabbits and 7 patients. The red cell survival of rabbits were determined by both radioactive and non-radioactive method. There were fairly good agreements. In the clinical study, the half-lives of  $^{50}\text{Cr}$  labeled red cells of 7 patients ranged from 14–29 days. These data also showed good agreements with those of  $^{51}\text{Cr}$  labeled red cells determined on the same subjects.