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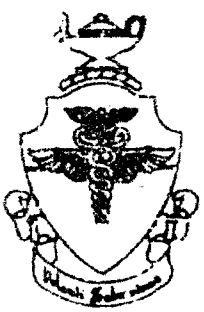

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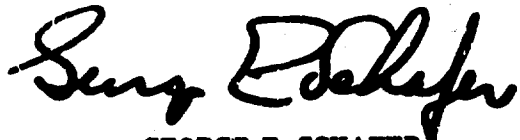
FOREWORD

This report was prepared in the Internal Medicine Branch, under task No. 775506. The work was accomplished between October 1967 and January 1968, and the paper was submitted for publication on 31 January 1968.

The Auto-Gamma spectrometer, model 5000, used in the study was manufactured by the Packard Instrument Company, Downers Grove, Ill.

This report has been reviewed and is approved.



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ABSTRACT

In scintillation spectrometry, there are methods for separating ^{59}Fe from ^{51}Cr activity, and ^{51}Cr from ^{125}I activity, but no standard method has been presented for the simultaneous measurement of ^{59}Fe, ^{51}Cr, and ^{125}I.

In this study, a well-type scintillation spectrometer and discriminator were used to determine the activity of these three isotopes in one sample. The procedure is based on the use of "contributory factors" which remain constant and can be used in the calculation of various blood parameters. The mixed sample was counted at each of three previously determined settings and corrected for background. Then, the net ^{59}Fe count equaled the total count at the ^{59}Fe setting; the net ^{51}Cr count equaled 10% of the count at the ^{59}Fe setting subtracted from the count at the ^{51}Cr setting; and the net ^{125}I count equaled the sum of 7.0% of the net ^{59}Fe count plus 7.0% of the net ^{51}Cr count subtracted from the count at the ^{125}I setting. The method can be used to separate the activity of any group of three or more gamma-emitting isotopes if their energy peaks are well separated.

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I. INTRODUCTION

In erythrokinetic studies, simultaneous measurements of several blood parameters are required. To measure all parameters, it is necessary to use three gamma-emitting radioisotopes—^{59}Fe, ^{51}Cr, and ^{125}I. Methods have been developed for separating ^{59}Fe and ^{51}Cr activity in the same sample (1), and ^{51}Cr and ^{125}I in the same sample (2); however, no method has been devised for a sample containing ^{59}Fe, ^{51}Cr, and ^{125}I. The peak gamma energies of these isotopes are well separated and suitable windows can be determined for measuring activity on the gamma spectrometer using appropriate discriminator settings. This report describes a standard procedure for simultaneous measurement of ^{59}Fe, ^{51}Cr, and ^{125}I, using these windows and appropriate correction factors.

II. MATERIALS AND METHODS

Iron-59

The ferric chloride ^{59}Fe containing benzyl alcohol, 0.9%, as a preservative and NaOH or HCl as necessary for pH adjustment was used as a sterile solution. This solution had a specific activity of approximately 10 mc./mg., with approximately 30 μc. of ^{59}Fe per milliliter of solution. Iron-59 has principal photopeaks of 1.10 Mev and 1.29 Mev and a physical half-life of 45 days.

Iodine-125

Iodine-125, buffered with sodium bicarbonate and containing 0.45% NaCl, was used as a sterile, pyrogen-free solution of radioiodinated human serum albumin. Benzyl alcohol, 0.9%, was present as a preservative. The solution had a specific activity of approximately 37.5 mc./mg. and approximately 10 μc./ml. of solution. ^{125}I has a principal photopeak of 35.4 kev gamma and a physical half-life of 60 days.

Chromium-51

Radiochromatic ^{51}Cr, containing sodium bicarbonate (1.0 mg./ml.) and NaOH or HCl for pH adjustment, was used as a sterile, buffered solution. This solution had a specific activity of approximately 42.7 mc./mg. and approximately 215 μc./ml. of solution. ^{51}Cr has a principal photopeak of 0.320 Mev gamma and a physical half-life of 27.8 days.

Instrument

The Auto-Gamma spectrometer used in the study had a 3- by 3-in. well-type NaI scintillation crystal. The well dimensions were $2\,^{1}/_{32}$ in. by $2\frac{1}{8}$ in. The photomultiplier tube multiplied incident photon energies to the 11 dynode. The instrument has been calibrated for full-scale energy of 1.0 Mev at a gain setting of 40%. Additional calibrations were made for settings of 0.5 Mev, 2.0 Mev, and 4.0 Mev with gains of 80%, 20%, and 10%, respectively.

Working solutions

Working solutions of the ^{51}Cr, ^{59}Fe, and ^{125}I were made by diluting quantities of the isotopes with sufficient sterile saline so that 0.5 ml. of solution yielded radioactivity of

approximately 10,000 counts per minute when counted for one minute at the respective window settings. One-half ml. of each isotopic working solution was put into separate counting tubes and sufficient saline added to raise the volume to 1.5 ml. Mixtures consisting of equal volumes (0.5 ml.) of the ^{59}Fe and ^{51}Cr working solutions, the ^{59}Fe and ^{125}I working solutions, and the ^{51}Cr and ^{125}I working solutions were then put into counting tubes. Sterile saline (0.5 ml.) was added to each tube, bringing the total volume to 1.5 ml. Equal volumes (0.5 ml.) of each of the three working solutions were put into one counting tube, giving this mixed sample a total of 1.5 ml. There were, at this point, seven samples: ^{59}Fe alone; ^{51}Cr alone; ^{125}I alone; ^{59}Fe and ^{51}Cr mixed; ^{59}Fe and ^{125}I mixed; ^{51}Cr and ^{125}I mixed; and ^{59}Fe, ^{51}Cr, and ^{125}I, mixed.

Settings for samples

The spectrums of the ^{59}Fe, ^{51}Cr, and ^{125}I samples were plotted by use of the Auto-Gamma spectrometer to determine the exact energy peak of each sample. The settings for the ^{59}Fe sample were: coarse gain set at 2 (0.2 Mev) fine gain at 3.5, and a window setting of 2%. With the discriminator setting at zero, a one-minute count was made. The discriminator setting was then set at 20 and another one-minute count taken. This procedure was repeated until, raising the discriminator setting by increments of 20, the one-minute counts reached a peak, then began decreasing until negligible. The same procedure was followed using the ^{51}Cr sample and the ^{125}I sample with changes being made in the instrument dial settings. The settings for ^{51}Cr were as follows: coarse gain of two (0-2 Mev) fine gain of 2.3, and a discriminator setting of 20 kev. The discriminator setting was raised by increments of 10. The dial settings for the ^{125}I sample were as follows: coarse gain of 8 (0 to 0.5 Mev), fine gain of 7.0, and a window setting of 2%. The discriminator setting was raised by increments of 10. The results of the one-minute counts were then plotted with the counts per minute as the ordinate and the instrument discriminator settings as the abscissa (fig. 1).

III. RESULTS

Determination of contribution factors

The seven isotope samples were counted for one minute at the ^{59}Fe setting, the ^{51}Cr setting, and the ^{125}I settings with all counts being corrected for background. The results of these counts appear in table I.

It is observed that with the ^{59}Fe sample, approximately 10% of the count at the ^{59}Fe setting appeared at the ^{51}Cr setting and 7.5% of the count appeared at the ^{125}I setting. When the ^{51}Cr sample was counted, no count appeared at the ^{59}Fe setting, but 7% of the count at the ^{51}Cr setting appeared at the ^{125}I setting. It may also be observed that when the ^{125}I sample was counted, no count appeared at the ^{59}Fe or ^{51}Cr settings. From these observations, contribution factors were derived, as shown in table II.

Separation of isotope activity

Mixed sample of iron-59 and chromium-51. The sample was counted at both the ^{59}Fe and ^{51}Cr settings and corrected for background. The net ^{59}Fe count equaled the total count at the ^{59}Fe setting. The net ^{51}Cr count equaled 10% of the count at the ^{59}Fe setting subtracted from the total count at the ^{51}Cr setting.

Mixed sample of iron-59 and iodine-125. The sample was counted at both the ^{59}Fe and ^{125}I peak and corrected for background. The net ^{59}Fe count equaled the total count at the ^{59}Fe settings. The net ^{125}I count equaled 7.5% of the count at the ^{59}Fe settings subtracted from the total count at the ^{125}I settings.

Mixed sample of chromium-51 and iodine-125. The sample was counted at both the ^{51}Cr and ^{125}I settings and corrected for background. The net ^{51}Cr count equaled the total count at ^{51}Cr settings. The net ^{125}I count equaled 7% of the count at the ^{51}Cr setting subtracted from the total count at the ^{125}I setting.

Mixed sample of iron-59, chromium-51, and iodine-125. The sample was counted at the

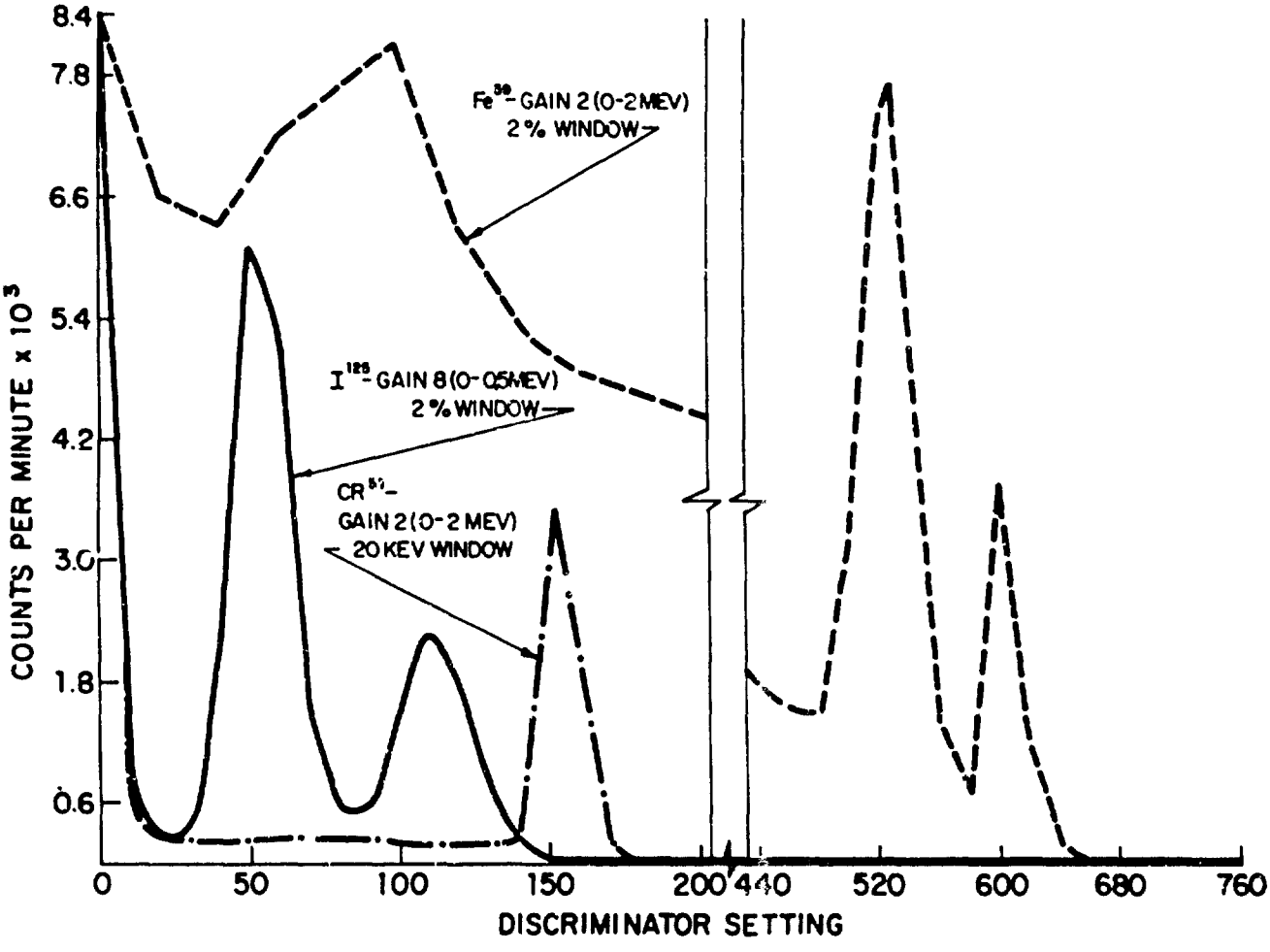

FIGURE 1

Results of one-minute counts of isotope samples using a gamma spectrometer.

TABLE I

Net counts at selected settings for three isotopes

Sample	Setting for ^{59}Fe	Setting for ^{51}Cr	Setting for ^{125}I
^{59}Fe	10,059	978	756
^{51}Cr	0	11,456	820
^{125}I	0	0	7,936
^{59}Fe and ^{51}Cr	10,171	12,587	1,566
^{59}Fe and ^{125}I	9,980	954	8,818
^{51}Cr and ^{125}I	7	11,291	8,658
^{59}Fe, ^{51}Cr, ^{125}I	10,081	12,283	9,337

TABLE II

Contribution factor at selected settings for three isotopes

Sample	Setting for ^{59}Fe	Setting for ^{51}Cr	Setting for ^{125}I
^{59}Fe	100%	10%	7.5%
^{51}Cr	0%	100%	7.0%
^{125}I	0%	0%	100%

^{59}Fe setting, ^{51}Cr setting, and ^{125}I setting and corrected for background. The net ^{59}Fe count equaled the total count at the ^{59}Fe setting. The net ^{51}Cr count equaled 10% of the count at the ^{59}Fe setting subtracted from the count at the ^{51}Cr setting. The net ^{125}I count equaled the sum of 7.5% of the net ^{59}Fe count plus 7.0% of the net ^{51}Cr count subtracted from the count at the ^{125}I setting.

IV. DISCUSSION

One method commonly used to separate ^{59}Fe and ^{51}Cr is the "Z" factor method. This method is based on the principle that the amount of radiation due to ^{59}Fe at the iron peak is directly proportional to the amount of radiation due to ^{59}Fe at the chromium peak. The "Z" factor is determined by dividing the number of counts due to radiation from the iron standard, at the ^{51}Cr peak, by the number of counts at the ^{59}Fe peak. The counts at the iron peak are then multiplied by this factor and this product is subtracted from the counts at the ^{51}Cr peak to give the net counts due to ^{51}Cr.

We have expanded on the "Z" factor concept to make possible the separation of ^{59}Fe, ^{51}Cr, and ^{125}I. As a first step in this process, contribution factors for $^{59}Fe/^{51}Cr$, $^{59}Fe/^{125}I$, and $^{51}Cr/^{125}I$ are determined. Using the $^{59}Fe/^{51}Cr$ factor, the ^{51}Cr activity is determined. Using the $^{59}Fe/^{51}Cr$ factor and the $^{51}Cr/^{125}I$ factor, the ^{125}I activity is determined. This method can be used to separate any group of three or more gamma-emitting isotopes if their energy peaks are well separated.

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