Standardized annular assay of dual radioisotopes on a well type sodium iodide crystal

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SYNOPSIS A procedure is described for the determination of the separate amounts of two gamma-emitting radioisotopes present simultaneously in large liquid volumes using an annular cell placed over a standard well-type crystal of sodium iodide and a reference source of ^{137}Cs . This sensitive technique is illustrated with particular reference to the double radioisotope urinary excretion test, using orally administered $^{57}\text{CoB}_{12}$ bound to human gastric juice and $^{58}\text{CoB}_{12}$ simultaneously, for the differentiation between patients with intrinsic factor deficiency and other causes of vitamin B_{12} malabsorption.

The problem of determining the separate amounts of two radioactive isotopes emitting radiations of different energies in a sample containing both isotopes simultaneously has been discussed by Veall and Vetter (1958) and may be considered with reference to the assay of blood samples containing ^{51}Cr and ^{59}Fe .

Blood samples are often restricted to about 5 ml which may be readily accommodated in individual test tubes and counted in the well of a 2 in. crystal of sodium iodide. The procedure for ^{51}Cr and ^{59}Fe mixtures using a single-channel instrument is usually one in which each test tube containing blood and standard solutions containing each radioisotope separately are assayed under conditions of discriminator bias or channel width to detect mainly the 1.1 and 1.29 Mev gamma rays from ^{59}Fe . The samples and standards are then recounted with appropriate conditions of discriminator bias or channel width to detect most of the 0.32 Mev gamma rays from ^{51}Cr . After correcting for background contributions the solving of two simultaneous equations, incorporating the measurements under the two sets of counting conditions, provides the individual amounts of each radioisotope present in the blood samples.

When assaying urine from a patient a volume of about a litre is usually available from a 24-

hour urine collection, and Bell (1965) has shown that better counting performance for a range of six radioisotopes may be obtained by assaying 400 ml volumes in an annular cup placed over the crystal compared with assaying 5 ml in the well of the crystal.

However, it is not always convenient to accommodate 400 ml volumes in separate annular cups because of space and cost compared with test tubes. Hence one tends to count all the samples in the same annular cup, rinsing it out between samples in a fashion similar to the assay of β -emitting radioisotopes in a liquid sample β counter type M6¹. This procedure is straightforward when counting a single radioisotope in the sample, but in order to minimize the number of times the annular cup has to be filled during the assay of samples containing two radioisotopes a standard procedure has been developed to ensure that the correct assay conditions are maintained.

This procedure is outlined with particular reference to the assay of ^{57}Co and ^{58}Co in urine arising out of the study of B_{12} absorption in patients using a dual isotope technique (Bell, Bridges, and Nelson, 1965), but it may be applied to any two gamma-emitting radioisotopes of suitable gamma ray energy difference. Standardization of the two assay conditions is obtained

Fig. Normalized variation in counting rate with increasing discriminator bias per 400 ml solutions of ⁵⁷Co and ⁵⁸Co in the annular cell and ¹³⁷Cs in a test tube in the well of the crystal using the integral mode of counting.

by using a test tube containing ¹³⁷Cs which emits gamma rays of energy 0.66 Mev compared with 0.12 and 0.81 Mev from ⁵⁷Co and ⁵⁸Co respectively.

The problem is to determine the percentage of the ⁵⁷CoB₁₂ bound to intrinsic factor, and ⁵⁸CoB₁₂ doses excreted in the urine following the simultaneous oral administration of 0.25 μCi of these radioactive compounds to patients.

Initial Setting Up

The high voltage supply to the photomultiplier tube viewing the sodium iodide crystal and amplifier gain are adjusted to give the integral spectrum for 400 ml of ⁵⁷Co in the annular cup between 5 and 30 volts (V) discriminator bias. Using these values of high voltage and amplifier gain, the integral spectra for 400 ml ⁵⁸Co in the annular cup and 0.2 μCi ¹³⁷Cs in a 1.0 ml volume sealed in a test tube of 3/8 in. diameter assayed in the well of the crystal are also obtained. The counts registered on an associated scaler are normalized to 100% at 5V discriminator bias as shown in the Figure. The actual counts detected from the ¹³⁷Cs standard source in 100 seconds in the well of the crystal are noted both at 5 and 35V.

Assay of ⁵⁷Co and ⁵⁸Co

The sequence of counting is carried out as indicated in the Table with the ¹³⁷Cs standard source assayed in the well of the crystal, and the samples and separate standards are made up in 400 ml water containing 10% of the ⁵⁷Co and ⁵⁸CoB₁₂ doses respectively assayed in the annular cup, and the averages of the two 300-second counting periods are considered for the sample counts at discriminator settings of 5 and 35V. The ¹³⁷Cs standard need only be counted for 100 seconds since the quantity of radioactivity is chosen to provide a high counting rate.

Calculation

Consider the Table where the data obtained at 5 and 35V discriminator bias settings are presented in symbolic form together with an example using data obtained from the investigation of a patient. The small letters indicate the appropriate average observed counts in the counting periods. Steps 14, 15, and 16 may be omitted if the assay procedures are carried out under conditions in which the backgrounds do not vary greatly. These requirements are usually assisted by keeping the ¹³⁷Cs standard in a lead

Samples and Standards	Setting		Symbols		Example	
	Time (Sec)	Disc Bias (Volts)	Average Counts	Background Subtracted	Average Counts	Background Subtracted
1 ¹³⁷ Cs standard	100	5V	(d) ¹		(85615)	
2 Back round-400 ml water	300	5V	e		4287	
3 Background-400 ml water	300	35V	f		2359	
4 ¹³⁷ Cs standard	100	35V	(g)		(55892)	
5 Sample 400 ml	300	35V	n	n - f = N	3376	1017
6 Repeat 5						
7 Sample 400 ml	300	5V	m	m - e = M	16050	11763
8 Repeat 7						
9 ¹³⁷ Cs standard	100	5V	(d)		(85415)	
10 ⁵⁷ Co standard 400 ml (10%)	300	5V	a	a - e = A	28534	24247
11 ⁵⁸ Co standard 400 ml (10%)	300	5V	b	b - e = B	17712	13425
12 ⁵⁸ Co standard 400 ml (10%)	300	35V	c	c - f = C	11662	9303
13 ¹³⁷ Cs standard	100	35V	(g)		(55329)	
14 Repeat 3						
15 Repeat 2						
16 Repeat 1						
17 Measure total volume of urine excreted, let this be Tml						1,020 ml

Table Standardized counting procedure

¹The counts observed from the ¹³⁷Cs are shown in brackets (d) and (g) to indicate that they are used only to check the instrument settings and are not used in calculating the percentage of the ⁵⁷Co and ⁵⁸Co doses excreted.

shield, with walls at least 2.5 cm thick, when not required.

Since the 35V discriminator bias excludes the gamma rays from ^{57}Co the simultaneous equations to be solved are simplified. Thus the percentage of the $^{58}\text{CoB}_{12}$ dose excreted is given by

$$x = \frac{N T}{40C} . \text{ The percentage of the } ^{57}\text{CoB}_{12} \text{ dose}$$

$$\text{excreted is given by } y = \left(M - \frac{BN}{C} \right) \frac{T}{40A} .$$

The ratio of the amounts of ^{57}Co and ^{58}Co excreted is obtained by dividing y by x .

Using the figures quoted in the Table, the percentage of $^{58}\text{CoB}_{12}$ excreted is given by

$$\frac{1017 \times 1020}{40 \times 9303} = 2.8\% . \text{ The percentage of } ^{57}\text{CoB}_{12}$$

excreted is given by

$$\left(11763 - \frac{13425 \times 1017}{9303} \right) \times \frac{1020}{40 \times 24247} =$$

10.8%. The ratio of ^{57}Co to ^{58}Co equals 3.9.

It will be noted that the counts from the ^{137}Cs standard do not enter into the calculation of the fractions of ^{57}Co and ^{58}Co excreted in the urine. The ^{137}Cs standard serves only to indicate that the correct discriminator bias conditions have been properly selected in turn, and provides an immediate check for the inexperienced operator at the time of assay and from day to day due to its long half life of 30 years.

References

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