High counting rates from ^{125}I in radioimmunoassays

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SYNOPSIS Responses from one manual and four automatic gamma counters for assaying ^{125}I were compared. The resolution times quoted by manufacturers when applied in a standardized correction formula to obtain true counts from observed counting rates above 10^4 counts per second appeared to be inadequate. The need to investigate the errors associated with high counting rates for all instruments and electronic settings employed to determine ^{125}I in radioimmunoassays is emphasized.

The characteristics of a 60-day half-life and gamma and x-ray emissions of energy about 30 keV have made ^{125}I a popular choice in radioimmunoassays to determine pg/ml concentrations of substances in blood (Hunter, 1973). Each determination usually requires a fraction of a microcurie of a radioactively labelled compound to be measured in a well type scintillation counter set to detect ^{125}I . Automatic gamma counters capable of assaying over 200 samples in succession have been developed by different manufacturers who quote resolution times of one or two microseconds. Counting rates up to 10^4 counts per second (cps) can generally be accepted without the need for correction for the dead time of modern equipment. When counting rates (n) are in excess of this they should be corrected to obtain the true

counting rate (N) using the formula
$$N = \frac{n}{1 - nt}$$

where t is the dead time of the counting system (Veall and Vetter, 1958).

Adams and Zimmerman (1973) reported on dead time variations of gamma camera systems using a two-source method. This work prompted investigations of the response of five gamma counting systems used in different departments here which are sometimes used to determine the radioactivity in the eluate from Sephadex columns following labelling processes involving initial quantities of 1 or 2 millicuries of ^{125}I .

The construction of the lead shielding round the sodium iodide crystals in these counters did not readily permit the use of the two-source method. The counting rates and responses of the systems were therefore investigated using the technique of pro-

portional sources described by Faires and Parks (1958) for Geiger-Müller counters.

Method

An aqueous ^{125}I solution containing $8\text{ }\mu\text{Ci/ml}$ was prepared and accurate amounts containing $8\text{ }\mu\text{Ci}$ and 10 other amounts down to $0.08\text{ }\mu\text{Ci}$ were added to separate plastic tubes. The final volume in each tube was adjusted to 1.0 ml with distilled water. These were assayed inside test tubes which could be readily accommodated in the four automatic gamma counters and a manual counter under the electronic settings normally employed by the workers in the different departments for counting ^{125}I .

Results

Figure 1 displays the counting rates from the five counters against radioactivity in the samples. A thick curved line is used to help to identify the values obtained from the automatic gamma counter, SL, for which the manufacturers quote a resolution time of 1 microsecond for their scaler system. A regression line for this counter was determined from the counts of background and the five lowest ^{125}I samples and its linear extrapolation is also shown. Above 25 000 cps there is an increasing departure of the observed counts from the true counts obtained from the linear regression graph. Calculated dead times using the formula above yielded values 1.3 to 6.3 μs over the range of 25 000 to 74 000 cps for this system. The differences between the observed and true counts were expressed as a percentage error based on the observed counts and are given in figure 2.

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Fig 1 Variation in the observed counting rates from the assay of the ^{125}I samples in the five gamma counters. The extrapolated linear regression and the observed counts per second associated with the SL counter are identified by the thick lines.

Fig 2 The percentage errors associated with observed counting rates from the five gamma counters. The errors associated with quoted dead times of 1 and 2 microseconds up to 10^5 counts per second are indicated by the dotted lines.

A similar analysis was carried out for other automatic gamma counters, M1, M2, and a manual counter, EJ, for which the manufacturers quoted resolving times of 1 microsecond. The automatic counter MP had a quoted resolution time of 2

microseconds. (Counters M1, M2, and SL were supplied by the same manufacturer.) The percentage errors associated with the observed counting rates were determined from the calculated regression line for each counter. These are shown in fig 2 together with the calculated errors associated with fixed dead times of 1 and 2 μs for comparison. The counting rates from the $0.48 \mu\text{Ci}$ ^{125}I sample used in calculations of the five regression lines ($r = 0.99$) yielded detection efficiencies for the MP, SL, M1, M2, and EJ counters of 43, 46, 55, 58, and 58 respectively.

Discussion

The counting rates obtained from radioactive samples depend on a number of factors including amounts of radioactivity and the energy of the emitted radiations, volume of sample, size, nature, and shape of detector, high voltage supply, amplifier gain and response time, discriminator bias and channel width and the resolution times of the scaler and timer. These contribute to the apparent overall dead time of the complete system.

At 10^4 cps the errors are less than 2% for the five systems as illustrated in figure 2. This is tolerable in radioimmunoassays when account is taken of the statistical and pipetting errors. The MP automatic gamma counter yielded percentage errors for ^{125}I in keeping with the calculated errors using the manufacturers' quoted resolution time of 2 μs and this appears to be constant to nearly 10^5 cps.

Above 30 000 cps the percentage errors for the assay of ^{125}I in the other counters begin to differ greatly from the calculated errors using the manufacturers' quoted resolution time of 1 μs . At 70 000 cps the errors for the M2, M1, EJ, and SL counters at 28, 35, 73, and 75% respectively are in excess of the error associated with a system having a 2 μs dead time and are about 3.7, 4.7, 9.7, and 10.1 times that of a system with a 1 μs dead time. Above 70 000 cps the differences are much greater.

This investigation shows that the manufacturers' quoted resolution times for individual electronic parts of their system can be misleading when attempts are made to correct observed counting rates. It is therefore important for counting rates above 10^4 cps that investigation of the response of the total counting system be carried out so that adequate corrections may be applied for the instruments and conditions normally used in departments for determining ^{125}I in radioimmunoassays.

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