**TECHNICAL NOTE**

Indium-114m in Dual-Nuclide Studies with Cr-51: Comparison with Indium-111

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Simultaneous radioassay of In-111 and In-114m, each in the presence of Cr-51, has been investigated. Presented here are data demonstrating the efficacy of using In-114m in a dual radioassay with Cr-51, a radioisotope currently used in many cellular research applications. In-111 ($T_{1/2}$, 2.81 days) has recently received attention in clinical studies, but has less than satisfactory physical characteristics for animal studies involving two emitters or long-term recirculation. Indium-114m ($T_{1/2}$, 50.1 days) permits long-term in vivo and in vitro monitoring, so that dual-nuclide studies on cell recirculation can be performed.


A dual-nuclide technique using In-111 and Cr-51 was recently used to investigate in vivo kinetics of total-body distribution of two lymphocyte subpopulations (/1). The time saved by this simultaneous determination was significant, but the assay of these two emitters presented some unexpected problems due to the summing characteristics of In-111 photons. Indium-114m was considered as an alternative tracer because of its 192-keV photon emission and its considerably longer physical half-life (2). Here we present data demonstrating the utility of In-114m in dual-nuclide studies.

**MATERIAL AND METHODS**

Radionuclides were counted in a 1.75- by 2-in. NaI(Tl) well crystal. The spectra were stored in a 1024-channel analyzer. The data were integrated over selected energy ranges to determine optimum window arrangements and cross contamination; background was subtracted before processing each spectrum. After determination of the optimum counting conditions dual radioassays were performed using an automatic gamma counter with a dual pulse-height analyzer.

**RESULTS**

Indium-114m possesses a relatively long half-life ($T_{1/2}$, 50.1 days) compared with that for indium-111, ($T_{1/2}$, 2.81 days).

Moreover, the In-114m radionuclide is essentially monoenergetic (192 keV) whereas In-111 shows two strong gamma emissions (172 and 247 keV) and a summation peak (~420 keV). Chromium-51 is a relatively long-lived radionuclide (27.8 days) displaying monoenergetic gamma emission at 320 keV. These differences in photon emission are illustrated in Fig. 1, which shows the pulse-height spectra for In-114m (panel A), In-111 (panel B), and Cr-51 (panel C). The photon emission peaks from In-114m and Cr-51 are monoenergetic and conveniently dissimilar for the simultaneous radioassay of a mixed sample.

Simultaneous radioassay of mixed tracer samples was performed using a dual pulse-height analyzer operating with optimal window selections: In-114m, 150–240 keV; In-111, 130–270 keV; Cr-51, 280–350 keV. Cross contamination from Cr-51 was significantly reduced at In-114m energies (8.2%) compared with that for In-111 (14.6%). To illustrate this difference, the energy spectra of In-114m plus Cr-51, and of In-111 plus Cr-51, are shown in Figs. 2 and 3, respectively. Indium-114m and Cr-51, being monoenergetic, give spectral peaks with little overlap (Fig. 2). In marked contrast, In-111 and Cr-51 display a complex spectrum with significant overlap of In-111 into Cr-51 energy levels (Fig. 3). This overlap occurs because In-111 emits two gammas almost simultaneously, and if both are caught in the crystal, the scintillations can add to generate a sum peak at 419 keV (3).

The presence of a sum peak complicates quantitative counting of In-111, since the magnitude of the sum peak is characteristically sensitive to detector geometry. Effects of sample volume and of source position on the In-111 spectrum were investigated. Peak counts were made at 172, 247, and 421 keV for different window widths in the range 150–480 keV. Counting rates were normalized to 100% for a 1-ml sample volume and for zero mm sample distance above the bottom of the well crystal. Sample volume was increased to 5.0 ml and/or elevated in stages to 5 mm above the...
well crystal. When the 421 keV sum peak was included in the window, sensitivity decreased as sample volume increased, and as the source position rose within the well (5–15%). This is because gamma emission is nonisotropic, and as crystal-to-source geometry improves the probability of two photons' interacting simultaneously in the crystal and being summed together is greatly enhanced. Volume and sample geometry effects were less pronounced (5%) when the sum peak was excluded from the 172-keV and the 247-keV peaks in the sampling window. Although this helped reduce inefficiency of coincidence detection, the problem was obviated by using In-114m.

DISCUSSION

The labeling of isolated cells with In-111 has been reported as a new technique with important applications in clinical research (4–6). Platelets (7,8) and leukocytes (9) from man and laboratory animals have each been successfully radiolabeled and then visualized in vivo in short-term circulation studies. Recently, murine T- and B-lymphocytes were radiolabeled with In-114m oxine or Cr-51 to study long-term in vivo cell-cell interaction during pathophysiologic processes (1). The advantages of In-114m in such studies is clearly demonstrated in Fig. 2: the lack of a sum peak makes separation of the In-114m photopeak from Cr-51 easy and makes In-114m ideally suited for dual radioassay of two cell populations. As reported (1), In-114m can form an oxine chelate and be incorporated into target cells in the same manner as In-111. These physical characteristics indicate that In-114m should be an attractive radiolabel in long-term recirculation studies when used either by itself to study cell traffic, or in combination with Cr-51 to investigate in vivo cell interaction. As with In-111 oxine, how-

**FIG. 1.** Pulse-height spectra of radionuclides. A: indium-114m gives prominent peak at 192 keV. B: indium-111 shows sum-coincidence peak at about 421 keV. C: chromium-51 has monoenergetic gamma peak at 320 keV.

**FIG. 2.** Pulse-height spectrum for indium-114m plus chromium-51. Peak separation is ideal for dual-label counting.

**FIG. 3.** Spectrum for indium-111 plus chromium-51, showing sum-coincidence peak and poor peak separation. Unfavorable for dual-nuclide studies.
ever, (10,11), a low concentration of In-114m (≤5 μCi per 10^8 cells) should be used to avoid target-cell damage.

REFERENCES

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