Experiment 17 DUAL LABEL EXPERIMENT

OBJECTIVE:

To illustrate the dual label counting procedure,

THEORY:

In recent years, the dual label experiment has become increasingly popular, particularly where metobolic processes are being studied. The term "dual label" refers to two different isotopes being present in the same sample. The most common pair used in this way is ^{3}H and ^{14}C; however, any combination of isotopes is permitted provided their endpoint energies are separated by 10-20 KEV. Without this much separation, the window in which the higher energy isotope is counted is much too small in that the count interval is too large for a given statistical accuracy to be reached. Figure 1 shows the spectral relationship of ^{3}H to ^{14}C under unquenched conditions. Their respective endpoint energies, 0.018 and 0.155 MEV, are indicated on the abcissa.

The window labeled A contains the ^{3}H spectrum and part of the ^{14}C spectrum. Hence, for a dual label sample count, ^{3}H and ^{14}C data will be mixed in the count obtained in channel A. Standardization procedures will be used so that the two components of the count in channel A can be unfolded.

The window labeled B contains only the high energy portion of the ^{14}C β^- spectrum.

Figure 2 shows a set of calibration curves for a dual label experiment. The curve labeled $^{14}C/^{3}H$ is a plot of decimal efficiency of ^{14}C counted in the window above the ^{3}H (A) window, or the ^{14}C decimal efficiency in the B window vs. external standard number. The ^{3}H labeled curve is a plot of the decimal efficiency of ^{3}H counted in the ^{3}H (A) window vs. the external standard number. The ^{14}C in ^{3}H curve is a plot of ^{14}C decimal efficiency in the ^{3}H (A) window vs. external standard number. The curve labeled R is a plot of the ratio of ^{14}C counts in A to ^{14}C counts in B vs. external standard number.

This ratio will be used to unfold the ^{14}C and ^{3}H counts in the A window. These calibration curves are formed with two sets of standards, both single labeled. For the case where ^{3}H and ^{14}C are the labels, one set of standards is ^{3}H labeled and the other ^{14}C labeled. The standards are counted and the data thus obtained is plotted as shown in Figure 2. The dual label samples are then counted in the two windows, A and B, and the external standard number is measured; all data is recorded as:

CPM in A = A_{cpm}

CPM in B = B_{cpm}

External standard number = Ex. Std. #

Using the external standard number, the following decimal efficiencies are measured.

decimal efficiency $^{14}C/^{3}H = e_1$

decimal efficiency ^{14}C in $^{3}H = e_2$

decimal efficiency $^{3}H = e_3$

The value for R corresponding to the external standard number is also noted. The calculation of the dpm values first involves unfolding the ^{3}H and ^{14}C data in channel A as follows:

$$R = \frac{^{14}C \text{ cpm in A}}{^{14}C \text{ cpm in B}}$$

$$B_{cpm} \cdot R = {}^{14}C \text{ cpm in A} \qquad (1)$$

$$\frac{B_{cpm} \cdot R}{e_2} = {}^{14}C \text{ dpm in A}$$

$$\frac{B_{cpm}}{e_1} = {}^{14}C \text{ dpm in B} \qquad (2)$$

and

$$A_{cpm} - B_{cpm} \cdot R = {}^{3}H \text{ cpm in A}$$

$$\text{Total } {}^{3}H \text{ dpm} = \frac{A_{cpm} - B_{cpm} \cdot R}{e_3} \qquad (3)$$

Equations (2) and (3) are to compute the dual label sample's dpm values and equation (1) permits data separation.

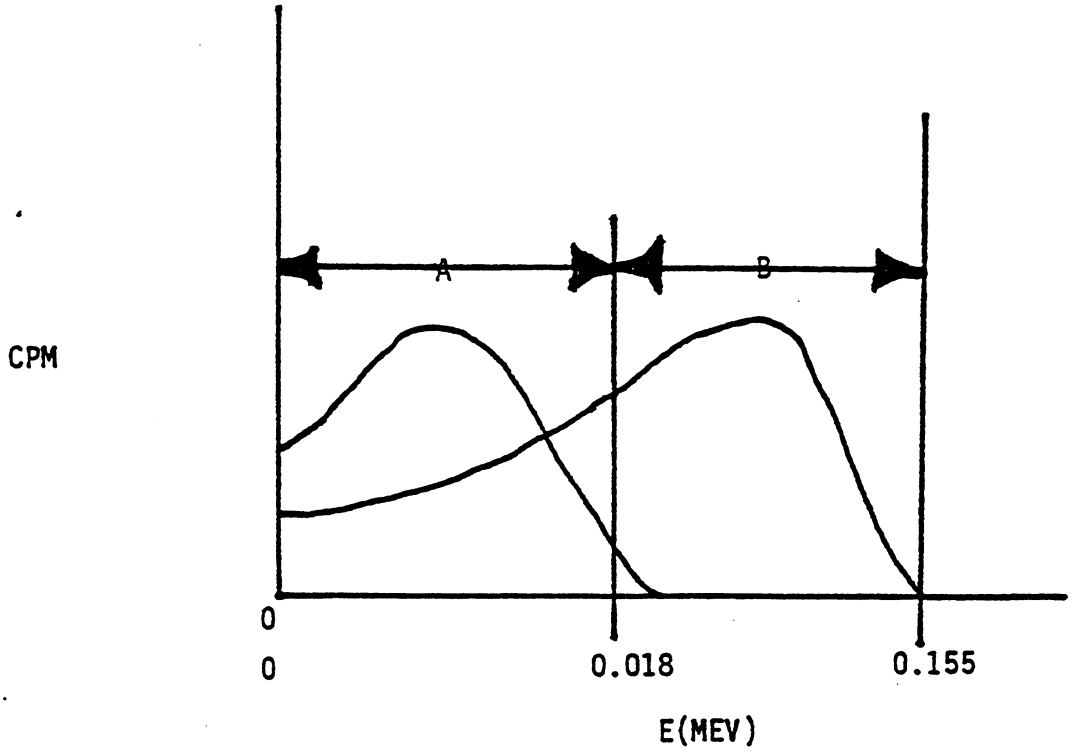
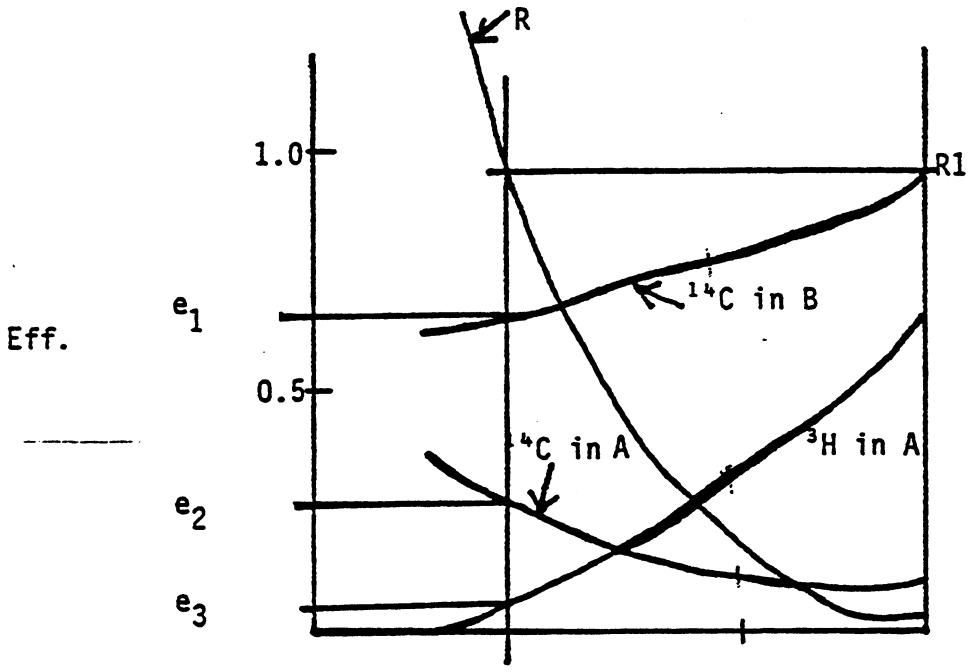


Figure 1



$$R = \frac{^{14}C \text{ in A}}{^{14}C \text{ in B}}$$

ESCR = Ext. Std. Channel Ratio $\left(\frac{4-3}{2-1}\right)$

Figure 2

A. The ^{14}C dpm may be obtained from <u>either</u> of the previous two equations: (1) & (2)

$$\frac{B_{cpm}}{e_2} \cdot R = {}^{14}C \text{ dpm} \qquad (1)$$

$$\frac{B_{cpm}}{e_1} = {}^{14}C \text{ dpm} \qquad (2)$$

Both equations should yield the same ^{14}C dpm. Which equation is best to obtain the most reliable ^{14}C determination and why?

B. Use the same ^{3}H and ^{14}C samples that you used in Expt. #4, i.e., samples 1A, 2B - 6B. Set A window equal to LLD-ULD = 0-150. Set B window equal to LLD-ULD = 150-400. Note that the ^{3}H spectrum cut off LLD with our setup is ∿ 120-150 and the ^{14}C spectrum cut off is ≈ 350. The actual amounts of radio activity, both for ^{3}H and ^{14}C, nitromethane should be recorded in your laboratory notebook. The following format is appropriate:

30,000 dpm ~2 sample work 30,000 dpm ~3 equiv ^{14}C

TABLE I

Sample #	^{3}H Activity DPM	^{14}C Activity DPM	Volume Scintillation Solutions*	CH_3NO_2 (ml)
1A-^{3}H	---	---	15	0.000
2B	---	---	15	0.010
3B	---	---	15	0.020
4B	---	---	15	0.030
5B	---	---	15	0.040
6B	---	---	15	0.050
1A-^{14}C	---	---	15	0.000
2B	---	---	15	0.010
3B	---	---	15	0.020
4B	---	---	15	0.050
5B	---	---	15	0.100
6B	---	---	15	0.150

*Same solutions as used in Expt. #4.

C. You have already measured and plotted in Expt. #7 the E for counting ^{3}H in the A window versus the ESCR (external standard channel ratio) as a function of CH_3NO_2 quencher. Plot and label e_3 versus ESCR.

D. Count ^{14}C samples 1A, 2B-6B in windows A and B (see Expt. 7 for description of windows A & B), and also determine the ESCR for each sample. From the ^{14}C in each sample, plot e_2 (E (efficiency) for ^{14}C in window A) and plot e_1(E for ^{14}C in window B) versus the External Standard Channel Ratios. Table III format is appropriate.

TABLE III

Sample #	^{14}C CPM A Window	Decimal Efficiency	^{14}C CPM B Window	Decimal Efficiency	ESCR
1A-^{14}C					
2B					
3B					
4B					
5B					
6B					

Also plot $R = \frac{^{14}C \text{ cpm in window A}}{^{14}C \text{ cpm in window B}}$ versus ESCR.

E. Obtain "cheerfully" some unknowns from the instructor!
Measure the cpm of the samples in windows A and B and also determine the ESCR for the samples. Calculate the ^{3}H dpm from equation (3) and the ^{14}C dpm from the two equations given in section A above. Also cite the estimated overall error in your final ^{3}H and ^{14}C dpm values.

TABLE IV

Sample #	CPM A Window	CPM B Window	ESCR
1			
2			
3			
4			
5			
6			