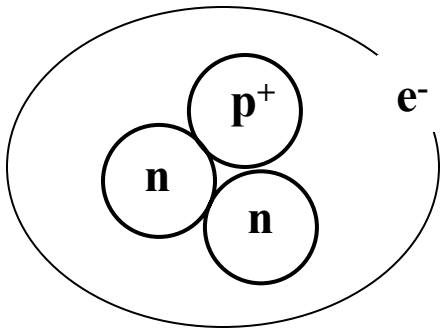


Advantages of Radioactivity

- sensitivity
- minor effects on chemical properties

Analytical Detection Limits

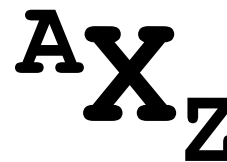
Technique	Limit of Sensitivity	
Spectrophometric	10^{15} molecules	10 nmole
Radiochemical		
^{14}C	10^{11} atoms	1 pmole
^3H	10^9 atoms	10 fmole
^{32}P	10^7 atoms	0.1 fmole



$^1\text{H}_1$ = hydrogen

$^2\text{H}_1$ = deuterium

$^3\text{H}_1$ = tritium



A = atomic mass
(neutrons + protons)

X = chemical symbol

Z = atomic number
(protons)

Isotopes

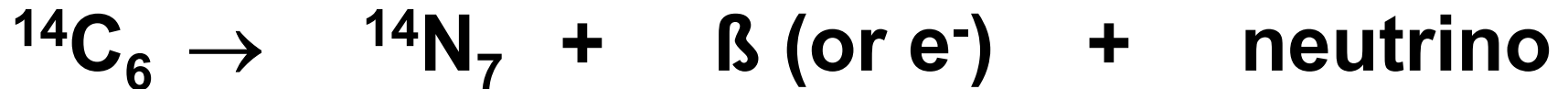
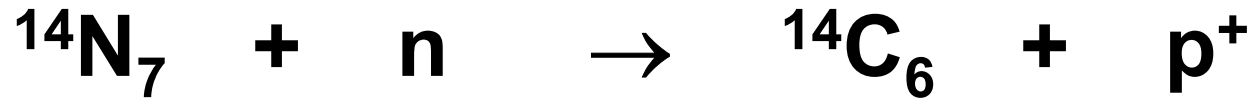
- species of the same chemical element with different atomic masses (ie, different numbers of neutrons)
- some isotopes are unstable and tend to rearrange or disintegrate
- disintegration involves the release of subatomic particles or electromagnetic radiation
- radioactive isotopes are called radioisotopes or radionuclides

Major Types of Radiation

α -particles	2p2n (He nucleus)
β -particles	electron (e^-)
γ -rays	photon ($h\nu$)

Only β - and γ -radiation are widely used in biological research.

Radioactive Decay



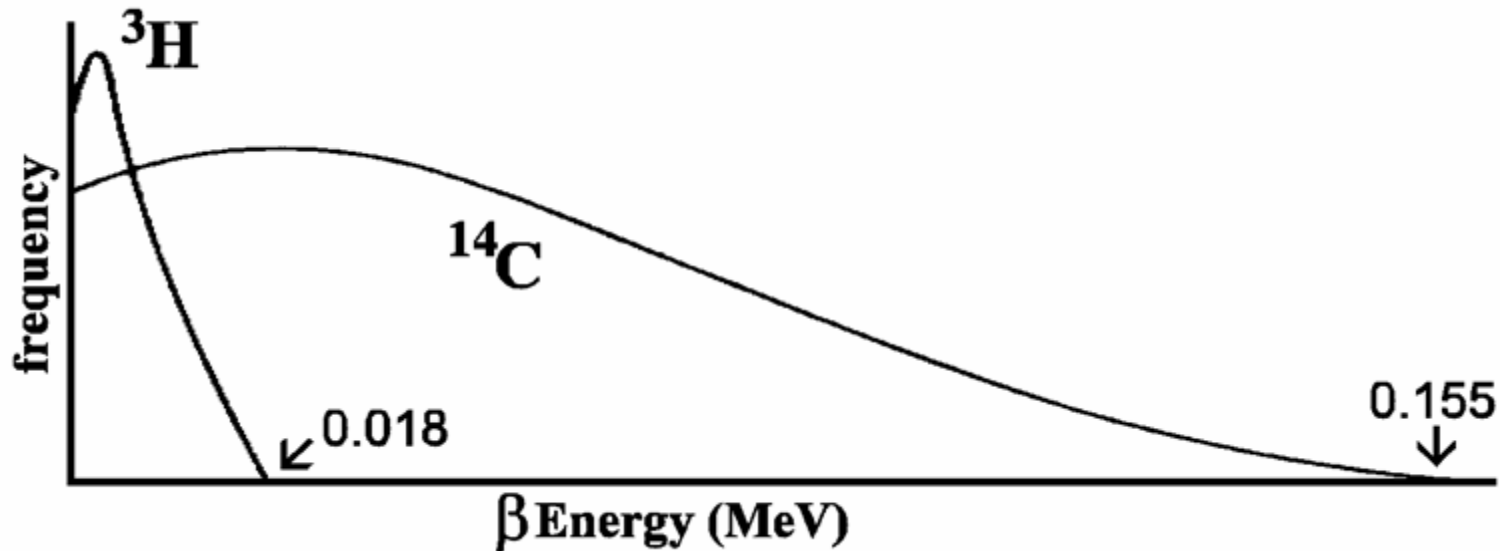
Radioisotopes characterized by:

- a defined decay rate (half-life)
- specific energy spectrum (ie, range)

Commonly Used β -Emitters

Isotope	* E_{avg}	* E_{max}	Half-Life
^3H	0.00555	0.018	12.3 years
^{14}C	0.05	0.155	5500 years
^{32}P	0.7	1.71	14.3 days
^{35}S	0.0492	0.167	87.1 days

*Energy expressed in MeV (mega electron volts)



Units of Radioactivity

dps (or dpm)	disintegrations per second (or minute)
cps (or cpm)	counts (detected disintegrations) per second or minute
becquerel (Bq)	official unit of measure (1 Bq = 1 dps)
curie (Ci)	earlier unit (1 Ci = 3.7×10^{10} dps)

Specific Activity

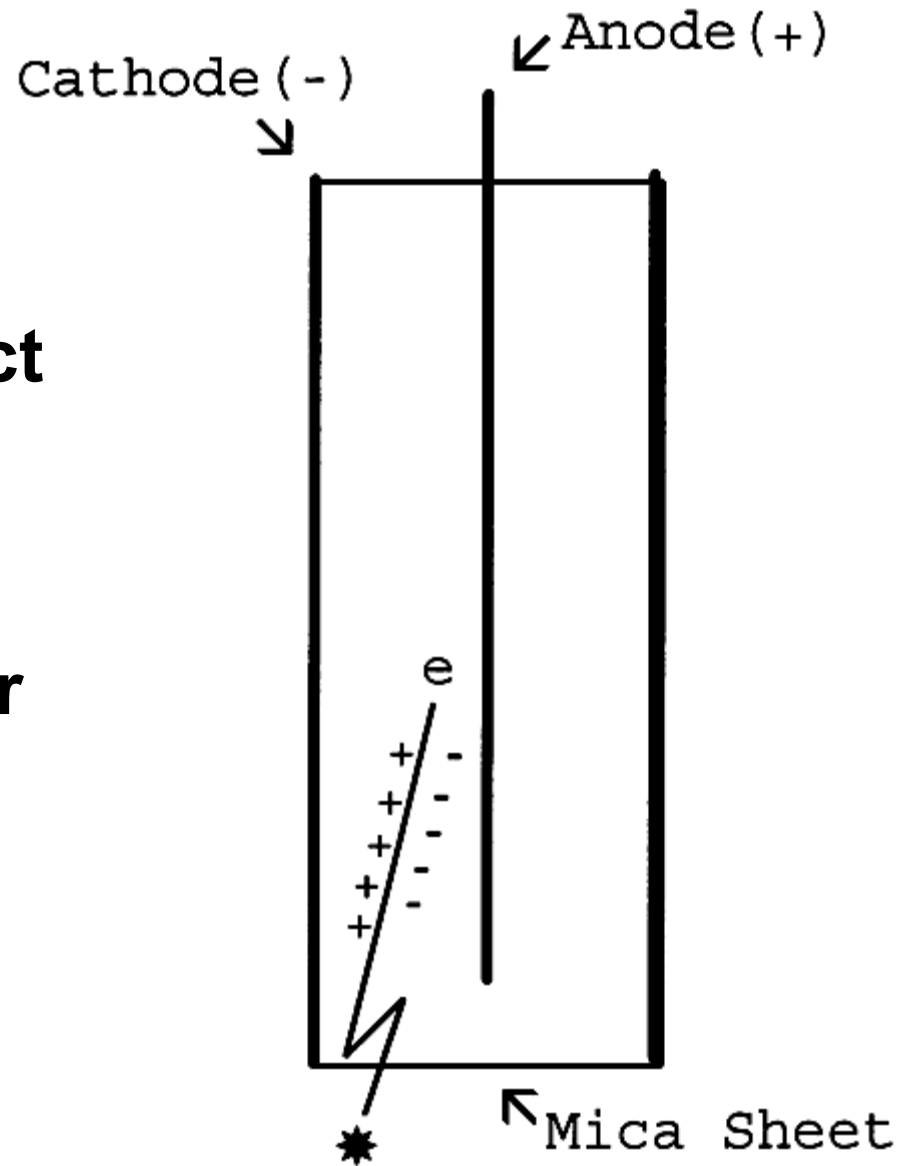
- **radioactivity per unit mass (eg., Bq/mmole)**
- **reflects mixture of radioactive and non-radioactive isotopes**

Radiochemical Purity

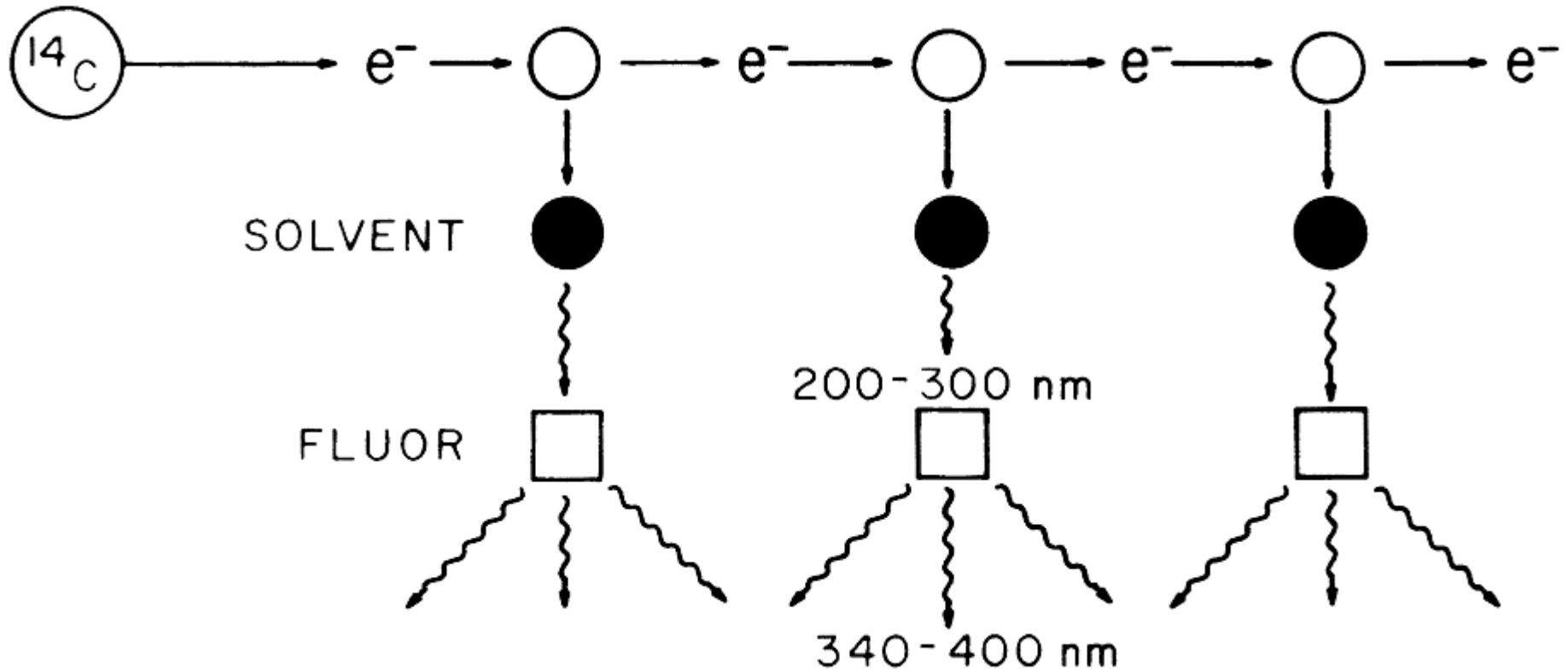
- **% of radioactivity in compound of interest**

Detection of β -Radiation

- all methods are indirect
- autoradiography
 - exposure to film or photographic emulsion
- Geiger-Mueller counter
 - ionizing radiation
- scintillation spectrophotometry



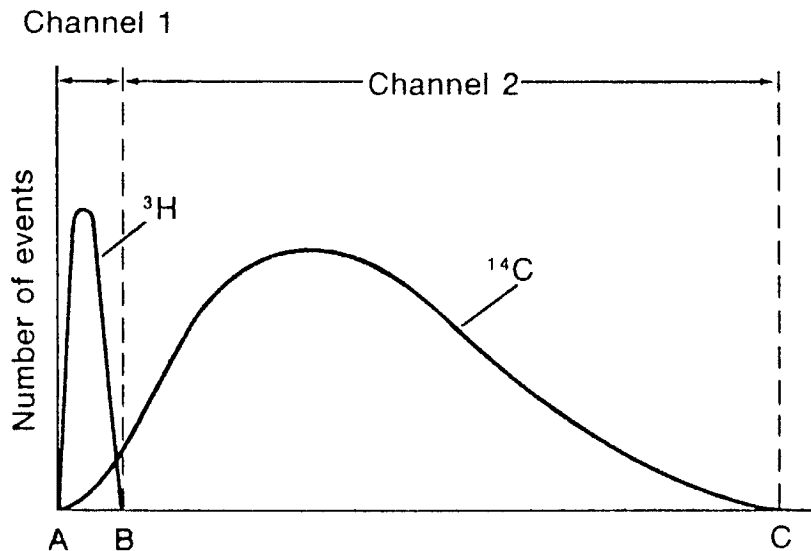
Scintillation Cocktail



- radioactive emission generates a flash of light
- light intensity proportional to energy of emission

Scintillation Spectrophometer

- determines # of decays (ie, flashes of light)
- discriminates energy of decay (ie, light intensity)
- records # of counts per energy level ('channels')

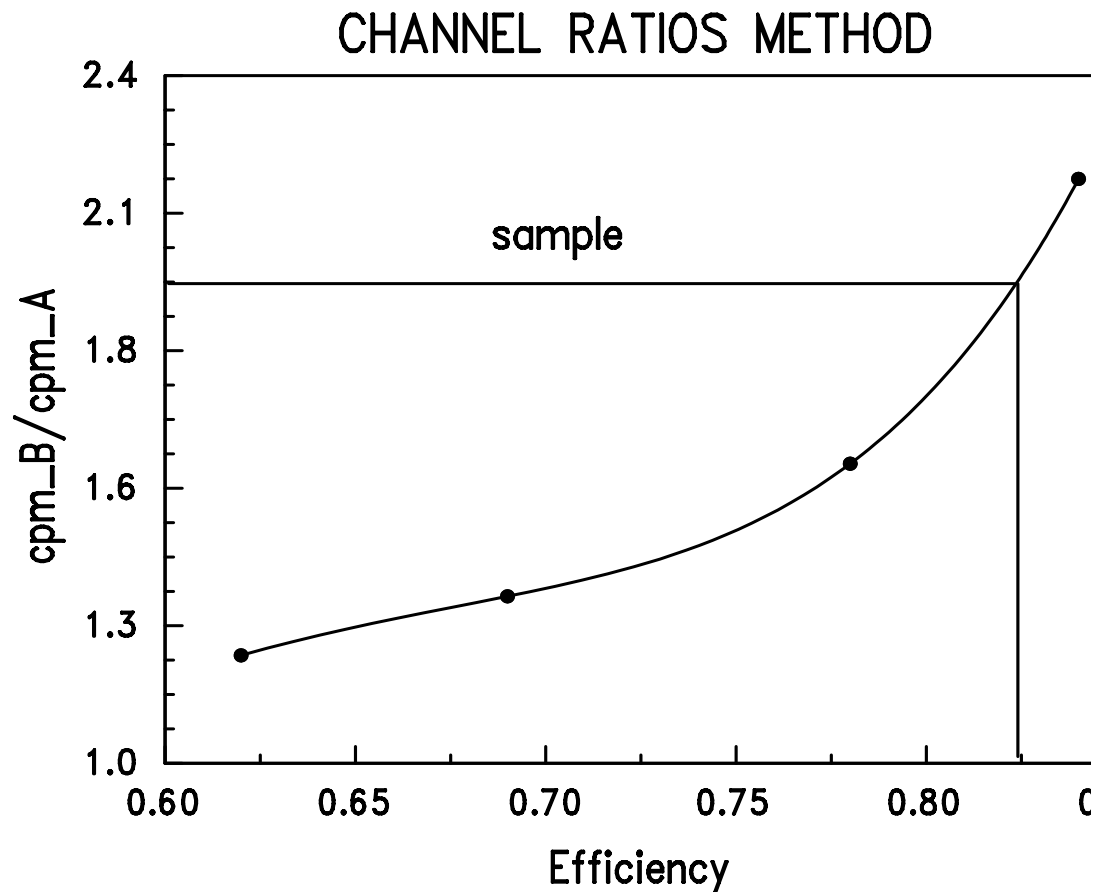
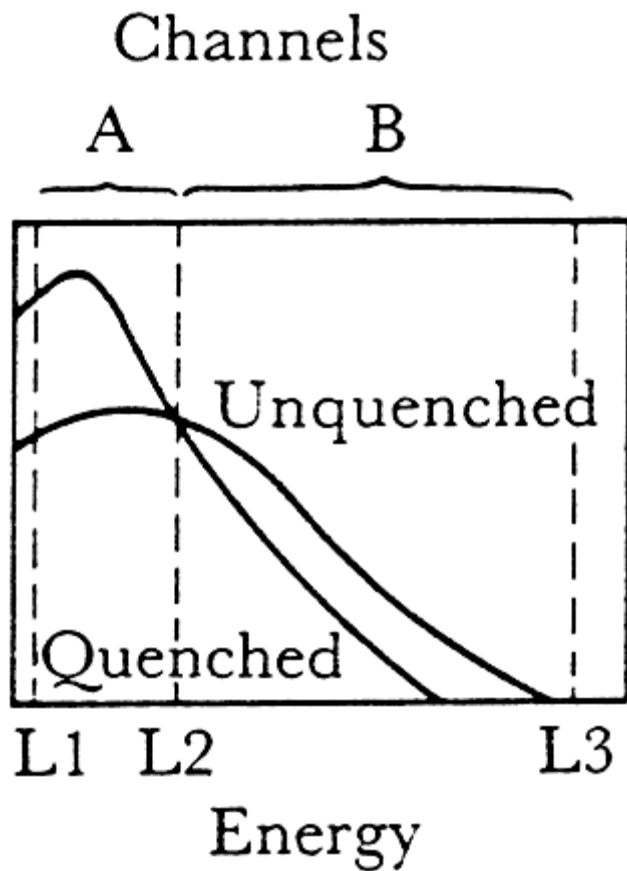


Practical Issues

- background
 - cosmic
 - natural
 - machine
 - sample
- accuracy
 - 10,000 counts \approx $\pm 2\%$ error
- efficiency

Efficiency of Counting

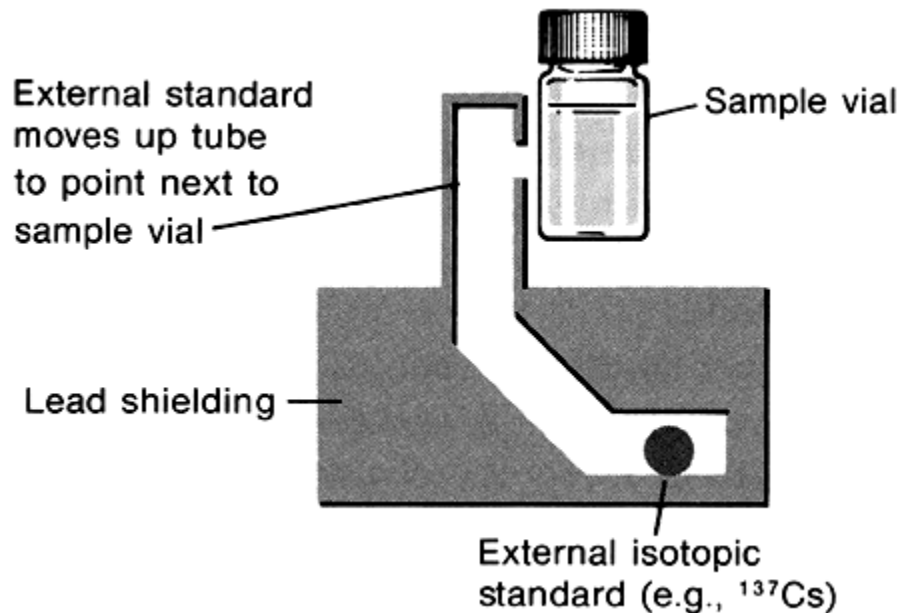
- defined as cpm/dpm
- quenching is the decrease in the transfer of energy from the β -particle to the phototube
 - chemical: reduction of fluorescence by substances in sample
 - color: absorption of fluorescence by substances in sample
- evaluation of quenching:
 - internal standards
 - channel ratios
 - external channel ratios



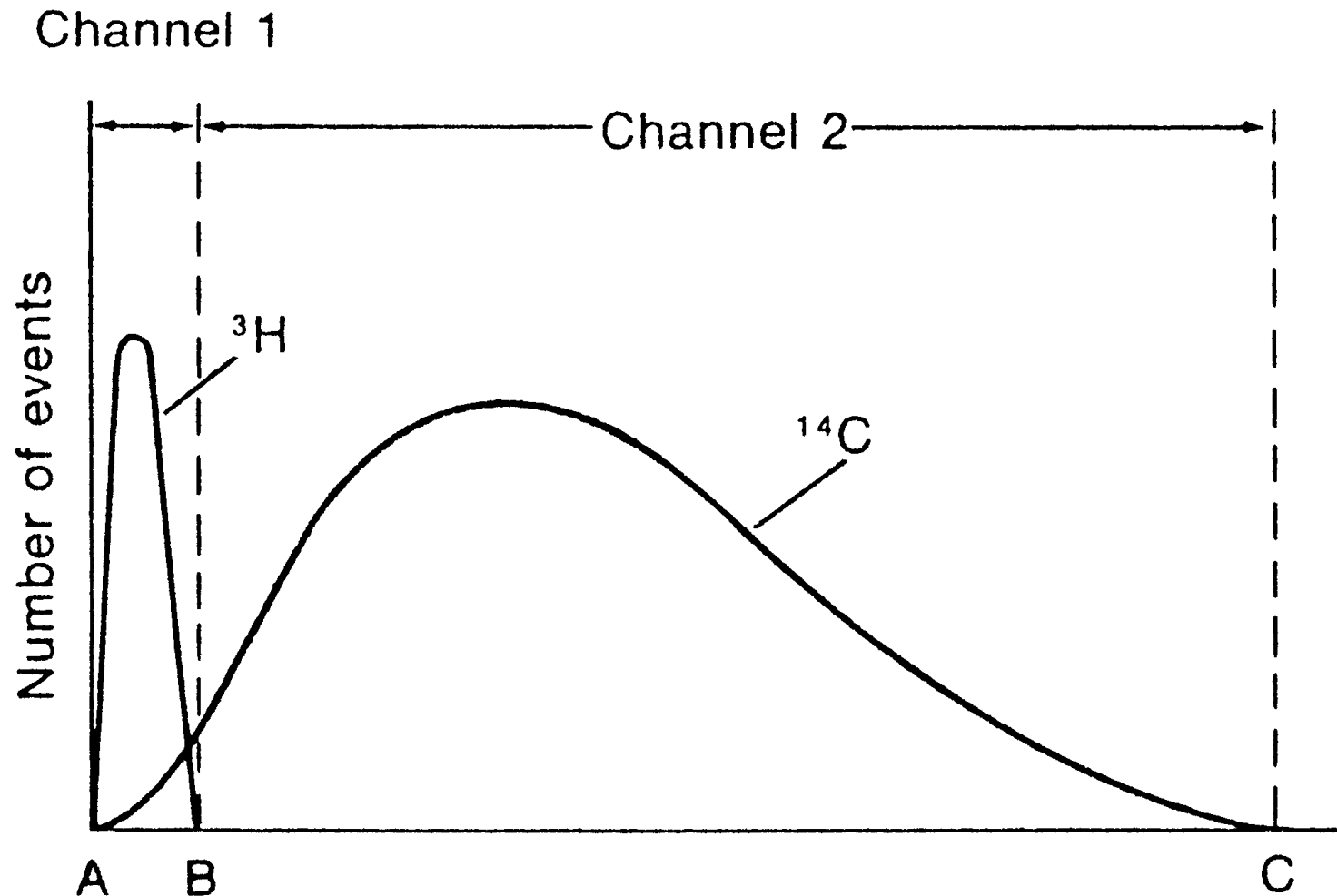
- quenching reduces energy of radioactive emissions
 - will alter channel ratios
- develop standard curve using known amount of radioactivity and quenching agent
 - plot channel ratios vs. efficiency

External Channels Ratios

- sample is exposed to external radiation source
- combines of principals of internal method and channel ratios method
- dpm calculated by instrument



Dual Labeling Experiments



Sample	Radioactivity (cpm)		Channel Ratios	
	Channel A	Channel B	B/A	A/B
Experimental	500	800		
³ H standard	16,840	25	0.0015	-
¹⁴ C standard	4250	10,200	-	0.416

³H in channel B = $(0.0015)(500) \leq 0.75$ cpm

¹⁴C in channel B = $800 - 0.75 \approx 800$ cpm

¹⁴C in channel A = $(0.416)(800) = 333$ cpm

³H in channel A = $500 - 333 = 167$

Total ³H = **167 cpm**

Total ¹⁴C = $800 + 333 = 1133$ cpm

Gamma-ray Counter

