

# LOW LEVEL TRITIUM COUNTING TECHNIQUES

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THE isotopic tracer technique is the most sensitive method available for the determination of interconversion and turnover of biologically active materials. The limiting parameters are the reaction rate, the duration of the reaction, and the amount of tissue or tissue derivative in the system. With increased specific activity of isotopic starting material, higher counting efficiency, and lower background, it becomes possible to study in detail the metabolic activity of a single cell or the distribution of a very small amount of a drug in a large animal.

It becomes apparent that the recent availability of tritium of high specific activity has increased its application as a biological tracer. In addition, there are many compounds which are impossible or tedious to label with other isotopes such as  $C^{14}$ , which may however readily be synthesized with tritium label either by means of catalytic hydrogenation of an oxidized derivative of the desired compound or by means of the WILZBACH<sup>1</sup> exchange method. There are various types of experiments such as the disposition or distribution of an intact drug or metabolite in which the high specific activity of the tritium-labeled preparation as well as the higher allowable human dose of the product make it preferable to  $C^{14}$ . Each isotope has specific advantages which must be considered in relation to a specific problem. Occasionally, a doubly labeled compound is used.

Tritium-labeled material may be counted in the dried state in a gas-flow proportional counter. The efficiency in this instance is found to be about 30% or the same as that for  $C^{14}$  at the  $C^{14}$  counting voltage. The background is about 35 counts/min. However, the high degree of self-absorption observed with tritium limits the sample size to a few  $\mu\text{g}$ . In the coincidence scintillation counter, efficiency of a toluene-soluble tritium compound in a glass vial is about 15–20% with a background of about 70 counts/min. Since much larger amounts of material can be counted by the latter method, we undertook to improve the tritium scintillation counting by lowering the background. A major component of the background was the fluorescence produced by the naturally occurring  $K^{40}$  in the glass vials. Silica has been used in phototube windows to reduce  $K^{40}$  background and silica vials have been employed in non-coincidence counting to reduce phosphorescence of glass detected by a

single phototube.<sup>2</sup> This suggested to us that silica vials would solve the  $K^{40}$  problem in coincidence counting. Vycor and silica vials were prepared by Mr. J. E. Fuller, Jr., American Instrument Co., Silver Spring, Maryland. The silica vial was made from Amersil clear fused quartz tubing (22 mm o.d.) which was closed and flattened. The other vials were of approximately the same diameter.

Samples were counted for 30 min in a Tri-Carb scintillation spectrometer. Into each vial were placed 5 ml of toluene containing 0.4% 2,5-diphenyl-oxazole and 0.01% *p*-bis [2-(5-phenyloxazolyl)]-benzene. After background counts were made, 0.01 ml of a toluene-soluble tritium standard was added to each vial and the efficiency of each sample was found to be essentially the same.

The results<sup>3</sup> are tabulated in Table 1. It can be seen that using a silica vial, a tritium counting efficiency of about 13% is obtained with a background of about 17 counts/min (10–50V) or 20% with a background of 37 counts/min (10V–∞). With  $C^{14}$ , a counting efficiency of 55% is found with a background of about 5 counts/min (10–50 V). The vials are easily decontaminated for re-use. Ultimately, it is hoped that an expendable vial containing no phosphorescence, fluorescence, or natural radioactivity will be developed.

TABLE 1  
*Comparative Background Counts with Various Vials*

	$H^3$ range $\approx 1300$		$C^{14}$ range $\approx 1000V$	
	10*–50V	50V–∞	10–50V	50V–∞
Commercial vial A	counts/min 52.6	counts/min 50.0	counts/min 7.2	counts/min 15.2
Commercial vial B	72.2	72.4	12.2	16.1
Vycor vial	40.3	32.9	9.3	6.0
Silica vial	17.4	19.1	4.6	6.1
$C^{14}$ standard 39,300 disintegrations/min	—	—	21,752	1552
$H^3$ standard 204,000 disintegrations/min	26,243	14,981	—	—

\* Pulse height range.

In a typical experiment involving the analysis of tissue for tritium content, about 50 mg wet weight of radioactive tissue are placed in a counting vial and the open vial is placed in a lyophilization apparatus. After it has been dried, the tissue is easily ground to a powder with a glass stirring rod and can be dissolved in Hyamine<sup>4</sup> at room temperature in a few minutes, with a minimal

amount of color production. By selecting proper voltage tap and discriminator settings, an overall efficiency of about 4% is obtained for tritium in tissue. The background is about 10 counts/min, half of which is contributed by the glass in the phototube window.

As a parenthetical observation from the viewpoint of the biochemist, I would like to point out that the Wilzbach technique makes it possible to label material of undetermined structure. The use of a scintillation solvent panacea such as the toluene-Hyamine mixture makes it possible to count entire tissues and reaction mixtures without purification. It is then possible to perform experiments without identifying either the starting material or the products. Let us hope that we may be spared the voluminous literature that could result from the combination.

#### REFERENCES

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