Liquid Scintillation Counting of C^{14} and H^3 in Plasma and Serum. (24102)

PHILIP S. CHEN, JR. (Introduced by Frederic C. Bartter)
Section of Clinical Endocrinology, Nat. Heart Inst., N.I.H., Bethesda, Md.

Liquid scintillation technics for counting low energy β-emitting isotopes have proven to be sensitive, simple and rapid. A basic requirement for such counting is to dissolve the radioactive material in a phosphor-containing organic phase, usually toluene. Several general solutions to the problem of counting substances of low solubility in toluene include (a) the incorporation of ethanol, for counting of aqueous phases and (b) utilizing the complexing ability of a quaternary ammonium base, Hyamine for counting CO_2(1) amino acids and proteins(2). In this report, a combination of these technics has been employed to determine the total serum content of C^{14} and H^3 with accuracy and rapidity.

Methods. Apparatus and materials. Solutions were counted in the Packard Tri-Carb Model 314-DC Liquid Scintillation Spectrometer with counting chamber set at $-8°C$. Carbon^{14} was counted at photomultiplier voltage Tap 6 (1060 volts) and Tritium at Tap 10 (1390 volts). The pulse-height discriminators were set at 10-100 volts. Efficiencies with C^{14} and H^3 using 5.0 ml of toluene-phosphor were 68% and 21% respectively. Hyamine was prepared by the method of Eisenberg(3). Commercial Hyamine, 10X, Rohm and Haas, Philadelphia [p-(diisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium chloride] was recrystallized 4 times from 4 volumes of toluene, dried, and converted to free base by shaking the chloride (96 g) with silver oxide (25.5 g) and water (1.8 ml) in methanol (100 ml) for exactly 10 minutes. After centrifugation, the supernatant was exposed to sunlight for several days and recentrifuged. The preparation was 0.97 M. *Recommended procedure.* To one ml of Hyamine in a counting vial (5-dram, Wheaton Glass Co., Millville, N. J.) is added 0.1 ml plasma or serum. The vial is then rotated to dissolve the plasma proteins. (Addition of Hyamine to protein may result in a protein coagulation which requires heat to dissolve.) To the vial contents is then added 0.5 ml ethanol and 5 ml toluene containing 0.6% diphenyloxazole (DPO) and 0.02% 1,4-di [2-(5-phenyloxazolyl)] benzene (POPOP). The vial is gently rotated to produce a homogeneous solution, cooled to the temperature of the counting chamber ($-8°C$) and counted at the appropriate voltage settings for C^{14} or H^3. An internal standard is then added and the observed count corrected for the overall quenching caused by the protein, water, ethanol and Hyamine content.

Results. Measurement of total activity in plasma or serum without extensive sample preparation demanded a method for rapid solution of the sample in a scintillating medium. Hyamine was a suitable complexing agent for the plasma proteins, but the technic has certain limitations.

The plasma protein concentration may vary from one sample to another, giving rise to slight variations in the degree of quenching. Superimposed on this variable is the quenching which may be caused by hemolysis. In Table I is shown the C^{14} count of 10 aliquots of 0.1 ml serum from one sample. The standard deviation was $\pm$ 1.10%, only slightly greater than the standard error of counting 14,000 counts which is $\pm$ 0.85%. However, in counting 10 different samples of serum containing equivalent activities, the standard deviation was $\pm$ 3.6%, considerably greater than the counting error of $\pm$ 0.5% in these counts (>40,000 counts). It was noted that hemolyzed serums counted 2-5% less than unhemolyzed serums.

Adequate correction for quenching is effected by the counting of an internal standard. Table II shows the counting of 0.1-0.4 ml volumes of serum containing C^{14} or H^3. Each serum sample was easily dissolved by 1.0 ml of Hyamine, but the larger samples required more ethanol to form a single phase. In these samples, the greater quenching resulted in an observed cpm/0.4 ml only 50% greater

TABLE I. C^{14} Counting of Serum.

Sample	No. samples	Avg observed counts/min.	Stand. dev. (counts/min.)	Stand. dev. (%)	S.E. counting (%)
.1 ml replicates	10	7276	80.2	1.10	.85
.1 " different	10	6347	229	3.6	.5

TABLE II. Liquid Scintillation Counting of C^{14} and H^3 in Serum.

Isotope*	ml serum	ml ethanol	C.P.M., −bkgd.†	Increment owing to addition of internal std.	Overall efficiency (%)	C.P.M./ml serum
C^{14}	none	none		10,630	68	
	.1	.5	4,367	5,326	34.1	87,200
	.2	1.5	5,318	3,296	21.1	85,900
	.3	2.0	6,362	2,494	16.0	90,200
	.4	2.5	6,473	1,918	12.3	89,800
H^3	none	none		35,940	21	
	.1	.5	6,442	6,276	3.67	369,000
	.2	1.5	8,200	4,116	2.40	358,000
	.3	2.0	9,663	3,316	1.94	349,000
	.4	2.5	10,181	2,403	1.41	380,000

* C^{14} counted at Tap 6 (1060 volts), 10-100 volt window; H^3 counted at Tap 10 (1390 volts), 10-100 volt window.

† Bkgd. C^{14} 18.0 cpm.; bkgd. H^3 47.2 cpm.

than the observed cpm/0.1 ml serum. This points to the definite limitation of sample size. Specific activities as corrected by internal standard (cpm/ml serum) were nevertheless constant as seen in Table II.

For 0.1 ml volumes of serum, the 34% efficiency for C^{14} and 3.7% efficiency for H^3 compares favorably with other methods of measuring total activity. Background counts in the scintillation counter were 18.0 cpm. and 47.2 cpm for C^{14} and H^3 respectively.

Previously described technics for determination of total C^{14} radioactivity are either accurate but of low efficiency (counting of infinitely thick liquid samples with thin-window flow counters) (4) or sacrifice accuracy for simplicity (plating methods). The latter technics require self absorption corrections which are often of poor reproducibility and electrostatic effects may seriously disturb counting of dried plasma samples with a windowless counter. We are not aware of any other simple technic for counting total H^3 activity in plasma.

Summary. A liquid scintillation technic for counting total plasma or serum activity of C^{14} and H^3 is described. The serum is complexed by the quaternary ammonium base, Hyamine and dissolved in phosphor-containing toluene with the addition of ethanol. Correction for quenching is effected by recounting an added internal standard after determination of the observed count. The method was shown to be rapid and accurate for various volumes of serum.

1. Passmann, J. M., Radin, N. S., Cooper, J. A. D., *Anal. Chem.*, 1956, v28, 484.

2. Vaughan, M., Steinberg, D., Logan, J., *Science*, 1957, v126, 446.

3. Eisenberg, F., Northwestern Univ. Conf. Liquid Scintillation Counting, 1958. Pergamon Press, N. Y. City.

4. Cotlove, E., *Fed. Proc.*, 1955, v14, 32.

Received March 26, 1958. P.S.E.B.M., 1958, v98.