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Jeffay H & Alvarez J. Liquid scintillation counting of carbon-14. Use of ethanolamine-ethylene glycol monomethyl ether-toluene.
Anal. Chem. **33**:612-15, 1961.
[Department of Biochemistry, College of Medicine, University of Illinois, Chicago, IL]

A method is described in which ^{14}C in a biological sample is oxidized to $^{14}CO_2$, trapped in ethanolamine, and counted in a liquid scintillation counter as a toluene solution of ethylene glycol mono-methyl ether. [The *SCI*® indicates that this paper has been cited in over 450 publications since 1961.]

Henry Jeffay
Department of Biological Chemistry
College of Medicine
University of Illinois
Chicago, IL 60612

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"In the 1950s, we were limited in doing radioactive tracer studies with ^{14}C by the problem of the self-absorption of weak β emissions in the Geiger-Müller counters. The invention of liquid scintillation counters for ^{14}C offered great hope for improving our ability to move quickly and to more accurately compare samples with very different specific activities. In those days, the biochemists followed the advice of the manufacturers and stayed away from water-containing' samples in order to reduce quenching and efficiency. Unfortunately, most biochemicals are not directly soluble in solvents suitable for liquid scintillation counting.

"We needed a method to do balance sheet studies on *in vivo* protein catabolism. We wanted to measure the total activity in large and small samples with low or high specific activities. We needed a method that would be linear (directly proportional to the true radioactive concentration) and independent of the amount or volume of unlabeled material in the sample. Other investigators tried preparing gels or suspensions. Those interested in counting proteins or tissues also tried dissolving them in an alkali compatible with toluene or dioxane solutions of scintillators. However, most biological samples had a very limited solubility in Hyamine (quaternary base), typical of the bases used. Oxidation methods were used, trapping the CO_2 in alkali. But often the carbonate precipitated out of the scintillation media when present in modest amounts. Also, most organic bases had limited capacity to dissolve large amounts of CO_2.

"Our own intuition led us to believe an oxidation would be a universal and useful method if a CO_2 trapping agent with a large capacity and solubility in toluene could be found. After many failures we tried using a polar organic solvent to increase the solubility of the base in toluene. For more than a year we tried, but finally gave up.

"One Sunday morning I was reading a feature story about life in a nuclear submarine, and the problems of staying submerged for long periods. They removed the respiratory CO_2 with ethanolamine because it was nonvolatile and stable, with a high capacity and instantaneous complete absorption of CO_2. furthermore, the CO_2 could be removed and pumped out of the submarine.) Immediately a light bulb flashed in my mind and I called my lab technician, Julian Alvarez. On Monday we found a sample of ethanolamine, tried it as a CO_2 trap with our oxidation system of a ^{14}C sample, and counted it with toluene and enough ethylene glycol monomethyl ether to obtain one phase. It worked. Working almost without stopping, both Julian and I obtained all the data we needed to prove the method was perfectly linear over a wide range of radioactive concentrations, and totally independent of the specific activity. The entire project was completed in a few days. Immediately we redesigned our protein catabolism experiments to incorporate this new measurement af ^{14}C.

"Several months later, I became convinced that we had a universal method for the liquid scintillation counting of ^{14}C. So I decided to publish it. Of course I was wrong. Bray's solution became popular.[1,2] Oxidation was not convenient for a large number of samples. Now we have other means of doing the job. I would guess that this paper has been highly cited because it was published at the beginning of the era when we changed From Geiger-Muller counting of radioactivity in tracer studies to the use of the now universally used technique of liquid scintillation counting. This change was made possible by the development of methods of the types reported in this paper."

1. **Bray G A.** A simple efficient liquid scintillalor for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**:279-85, 1960. (Cited 9,425 times since 1960.)
2.....Citation Classic. Commentary on *Anal. Biochem.* **1**:279-85, 1960. *Current Contents* (2):16, 10 January 1977.