

Chapter 13

The Determination of Tritium in the Presence of Iodine-125

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INTRODUCTION

For some time there has been a need for a reliable assay method for samples labelled doubly with ^3H and ^{125}I . A procedure utilising both a γ -counter and a liquid scintillation counter was proposed during discussion following a contribution to an earlier meeting in this series.¹ Nevertheless, our own investigations and those of others² have revealed certain difficulties and anomalies associated with this isotope pair and it was felt that a systematic study of the problem was justified. We have considered three distinct approaches, two of which are based on well-known techniques in radiochemical assay. The third is a new technique and has the advantage of requiring only a liquid scintillation counter. It relies neither on energy discrimination nor on decay of the relatively short-lived ^{125}I , but as the work is still at an exploratory stage this paper must be regarded as a preliminary progress report.

ASSAY METHODS

Method 1. Double instrument

A liquid scintillation counter may be used to detect and count the decay events of both ^{125}I and ^3H ; the former with high efficiency, the latter with moderate efficiency. Unfortunately, a considerable overlap of the pulse height spectra of the two isotopes (Fig. 1) renders energy discrimination an unreliable basis for an assay method. However, since ^{125}I emits weak γ -photons and X-rays in addition to energetic electrons, it is possible to determine the iodine activity using a γ -counter. If the γ counting efficiency for ^{125}I is assumed to be constant, then the following simple method should be applicable:

$$dpm_{\text{I-125}} = cpm_{\gamma} / e_{\text{I-125}(\gamma)}$$

$$dpm_{\text{H-3}} = (cpm_{\beta} - dpm_{\text{I-125}} e_{\text{I-125}(\beta)}) / e_{\text{H-3}(\beta)}$$

where cpm = net count rate (counts min^{-1}); dpm = activity (disintegrations min^{-1}); e = efficiency; γ = γ -counter; β = liquid scintillation counter.

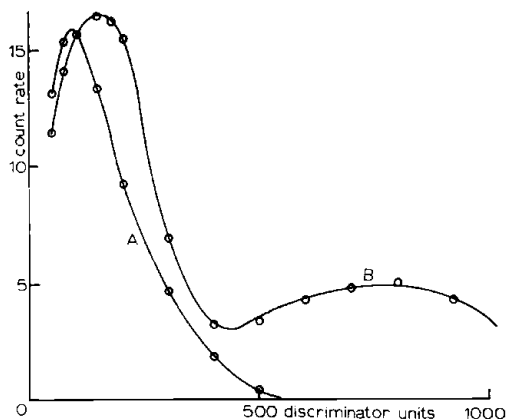


Fig. 1. Pulse height spectra of unquenched samples of ^3H (curve A) and ^{125}I (curve B) (0.8% butyl-PBD in toluene).

The variable liquid scintillation counting efficiencies are readily monitored by automatic external standard channels ratio.

In a variety of conditions, such a method yields results of high accuracy as typified by those for duplicate standards recorded in Table 1. The standards were counted once in the γ -counter and three times for each of four attenuation levels in the liquid scintillation counter. Further investigation showed that sample composition was important and very poor results were to be expected in certain circumstances. These included samples containing high levels of iodine activity and samples containing molecules incorporating heavy atoms.

The effect of heavy atoms cast doubt on the assumption that the γ counting efficiency was constant because it is well known that the absorption of γ -photons is a function of the atomic numbers comprising the absorbing material. These doubts were justified by showing that the γ counting efficiencies of sodium [^{125}I] iodide and [^{125}I] iodobenzene are reduced by successive additions of carrier (Figs. 2 and 3).

If the γ counting efficiency is reduced by a significant presence of high atomic number atoms, then the ^3H activity will be overestimated on two counts. First, the calculated iodine contribution to the β count rate will be low, thus exaggerating the ^3H contribution. Second, the γ -energy absorbed by the heavy atoms will be dissipated largely as photoelectric or Compton scattered electrons leading to an increase in the β count rate.

The principal failing of this method then is that the γ counting efficiency is not constant and, unlike liquid scintillation counting, automatic monitoring of counting efficiency is not available. For this reason we turned our attention to methods relying solely on liquid scintillation counting.

Table 1. Results obtained using a γ -counter and a liquid scintillation counter (Method 1).

Observed ^{125}I activity (dpm)	Recovery (%)	Attenuator setting	Observed ^3H activity (dpm)	Recovery (%)
16178	99.4	0.5	109384	99.8
			111639	101.9
			109522	100.0
16380	100.6		108889	99.4
			111133	101.4
			109022	99.5
16178	99.4	1.0	107607	98.2
			107603	98.2
			107479	98.1
16380	100.6		106156	96.9
			107010	97.7
			106879	97.6
16178	99.4	1.5	108134	98.7
			111353	101.0
			108885	99.4
16380	100.6		107449	98.1
			110659	101.0
			108196	98.8
16178	99.4	2.0	107678	98.3
			111324	101.6
			108480	99.0
16380	100.6		106922	97.6
			110558	100.9
			107719	98.3

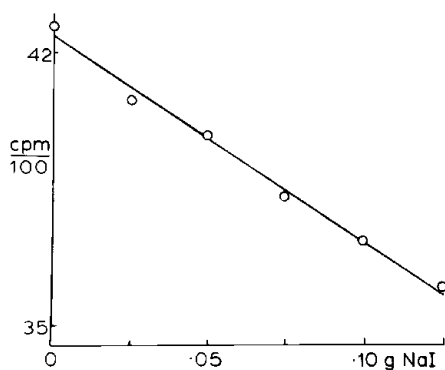


Fig. 2.

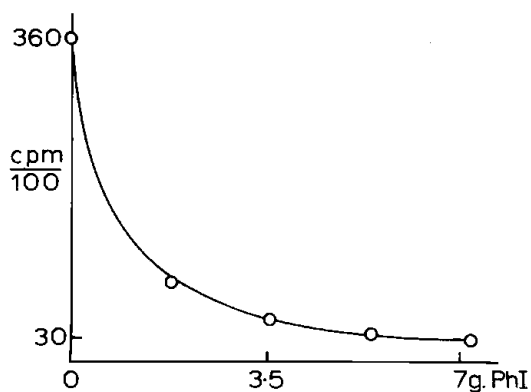


Fig. 3.

Fig. 2. The effect of adding sodium iodide carrier to an aqueous sample of ^{125}I counted in a polyethylene vial in the γ -counter.

Fig. 3. The effect of adding iodobenzene carrier to a toluene solution of [^{125}I] iodobenzene counted in a polyethylene vial in the γ -counter.

Method 2. Single instrument; iodine decay

Methods exploiting isotope decay are usually unattractive where many samples are to be routinely assayed. Such procedures necessitate storage of samples for a suitable period which in itself can be a problem where space is limited but more seriously leads to delayed results. This is at best frustrating and in certain laboratories, notably those undertaking clinical tests, intolerable. Nevertheless, we have looked at this approach for the sake of completeness.

The count rate of a double-labelled sample at day zero (cpm_0) is equal to the sum of the count rates due to the two isotopes:

$$cpm_0 = cpm_H + cpm_{I(0)} \quad (1)$$

Some few (t) days later the 3H decay will be negligible while that of the ^{125}I will be significant. The reduced count rate of the sample at day t will be given by

$$cpm_t = cpm_H + cpm_{I(t)} \quad (2)$$

The difference between the sample count rates at day t and at day zero is equal to the difference between the iodine count rates:

$$cpm_0 - cpm_t = cpm_{I(0)} - cpm_{I(t)} \quad (3)$$

By normal decay kinetics the following relationship exists:

$$cpm_{I(t)} = cpm_{I(0)}e^{-\lambda t} \quad (4)$$

where λ is the radioactive decay constant (days^{-1}) for ^{125}I . On changing the sign on both sides of Eqn. (4) and adding $cpm_{I(0)}$ to both, we have, on rearrangement,

$$cpm_{I(0)} - cpm_{I(t)} = cpm_{I(0)}(1 - e^{-\lambda t}) \quad (5)$$

Substituting from Eqn. (3) and rearranging gives

$$cpm_{I(0)} = (cpm_0 - cpm_t)/(1 - e^{-\lambda t}) \quad (6)$$

Thus, the count rate due to ^{125}I at day zero is obtained by dividing the difference between the two sample count rates by $1 - e^{-\lambda t}$. In practice the latter term may be tabulated for convenience. The ^3H count rate is then readily deduced from Eqn. (1). Table 2 records the results for a typical standard counted 2.67 and 5.67 days after the original count. Even after the longer period the results are unacceptable. Greater accuracy may be expected if several counts are taken during the decay period but this imposes even greater burdens on the routine analyst. An important point is that the decay period required to achieve a certain accuracy depends on the relative abundance of the two isotopes. Figure 4 illustrates plots of expected change in count rate (Δcpm) divided by the maximum error (E), predicted from the 90% confidence level, against time. The figure shows that any given accuracy is achieved after a shorter period for a sample rich in iodine than an equally active sample rich in ^3H .

Both from the point of view of accuracy and labour this method had little to recommend it.

Table 2. Results obtained using a liquid scintillation counter and ^{125}I decay (Method 2).

Period between counts (days)	Attenuator setting	Observed ^{125}I activity (dpm)	Recovery (%)	Observed ^3H activity (dpm)	Recovery (%)
2.67	0.5	17927	110.9	105742	96.6
	1.0	34708	213.2	53573	48.9
	1.5	27110	166.5	71826	65.6
	2.0	29178	179.2	59908	54.7
5.67	0.5	13723	84.3	118193	107.9
	1.0	16444	101.1	109438	99.9
	1.5	18489	113.6	102658	93.8
	2.0	12563	77.2	122570	111.9

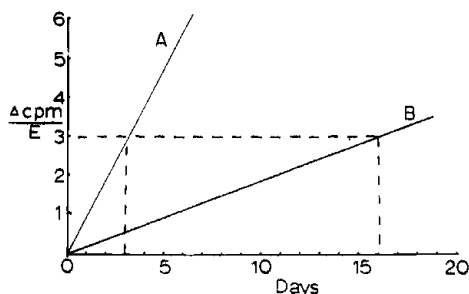


Fig. 4. Error curves for ^{125}I -decay in double-labelled samples. (A) $^3\text{H}:^{125}\text{I}$ activity ratio of 1:8. (B) $^3\text{H}:^{125}\text{I}$ activity ratio of 8:1.

Method 3. Single instrument; efficiency variation

This method exploits the effect of heavy atoms on the electron counting efficiency of ^{125}I . The addition of compounds containing such atoms should show two opposing effects on the count rate. First, there would be the normal quenching effect leading to

a reduction in counting efficiency. Second, the energy of the γ -photons absorbed by the additive would be dissipated largely by electron emission which would lead to an increase in counting efficiency. By careful control of the nature and quantity of the additive, the latter effect might be made predominant. The effect of such additives on ^3H would be to reduce the counting efficiency by quenching. The following scheme indicates how we may exploit these effects in an assay method. The count rate of a double-labelled sample may be expressed as follows:

$$cpm = e_I a_I + e_H a_H \quad (7)$$

where e = efficiency; a = activity; I = iodine-125; H = tritium.

Addition of a normal quenching agent would reduce the counting efficiency of each isotope. This leads to a second count rate cpm' :

$$cpm' = e'_I a_I + e'_H a_H \quad (8)$$

where $e'_I < e_I$ and $e'_H < e_H$.

Equations (7) and (8) would have an infinity of solutions in a_I and a_H since the quenching effects would be very similar for two isotopes with such similar energies. If, however, we are able to enhance the counting efficiency of the ^{125}I to e''_I , where $e''_I > e_I$, then a third equation may be written which can be uniquely solved with Eqn. (7) to yield a_H and a_I (Eqns. 10 and 11):

$$cpm' = e''_I a_I + e'_H a_H \quad (9)$$

In practice the accuracy of the solution will depend not only on the precision of our measurements but also on the relative magnitudes of the efficiency changes:

$$a_H = (e''_I cpm - e_I cpm') / (e''_I e_H - e_I e'_H) \quad (10)$$

$$a_I = (cpm - e_H a_H) / e_I \quad (11)$$

Provided a suitable additive can be found it is only necessary to count the sample and then recount immediately after adding the reagent. Then by reference to four calibration curves of efficiency against automatic external standard channels ratio for each isotope in the presence and absence of additive, four efficiencies are obtained and hence the activity of each isotope from Eqns. (10) and (11). The alternative to recounting the sample is to prepare two samples, one with additive, the other without.

In our laboratories, certain organotin compounds were readily available. This availability, coupled with the fact that the counting of ^{125}I in the presence of such compounds has already been studied,³ encouraged us to select them as our first additives. Initially we examined tetraphenyl tin, triphenyl tin acetate, tetramethyl tin and tetrabutyl tin. The first two were rejected because of poor solubility and our study was restricted to tetramethyl tin and tetrabutyl tin. The first stage was to determine the effectiveness of the reagents as γ - and X-ray absorbers by counting samples of ^{125}I iodobenzene containing increasing volumes of additive on the γ -counter. In terms of volume, tetramethyl tin is a more effective absorber than tetrabutyl tin (Fig. 5), but in terms of moles the behaviour of each additive is identical (Fig. 6).

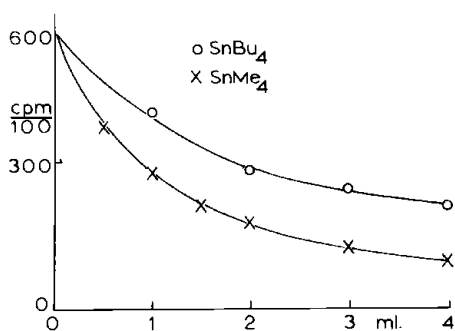


Fig. 5.

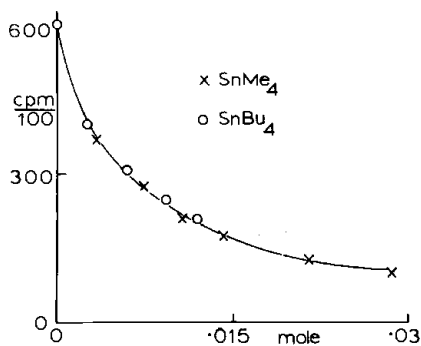


Fig. 6.

Fig. 5. The volume effect of adding organotin compounds to a toluene/butyl-PBD solution of [^{125}I] iodobenzene counted in glass vials in the γ -counter.

Fig. 6. The molar effect of adding organotin compounds to a toluene/butyl-PBD solution of [^{125}I] iodobenzene counted in glass vials in the γ -counter.

Absorption of the energy of the γ -photons and X-rays within the sample will lead to electron emission and this should be evident from changes in pulse height spectra from the liquid scintillation counter (Figs. 7 and 8). The unquenched, untreated spectrum exhibits a fairly sharp, intense low-energy peak. This may be attributed to Auger electrons while the broad, low-intensity, higher energy band is associated with internal conversion electrons and a few electrons arising from photoelectric and Compton scattering interactions between γ -photons and X-rays and the counting medium. The presence of additive nicely demonstrates both a quenching effect and an electron enhancement effect. The low-energy peak, which arises exclusively from electrons produced within the decaying atom, is clearly quenched since the peak intensity is considerably reduced. The higher energy region associated partially with X-ray and γ -photon interactions exhibits the expected increased intensity because of the increased electron density of the medium.

For quenched samples, the effects are similar, although there is an apparent increased intensity of the higher energy band due to the normal pulse height shift with impurity quenching.

The pulse height spectra for ^3H (Fig. 9) indicate that the effect of the additive is merely to reduce the counting efficiency in the normal way.

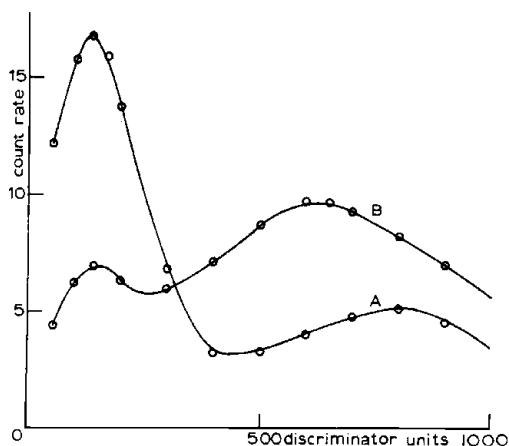


Fig. 7.

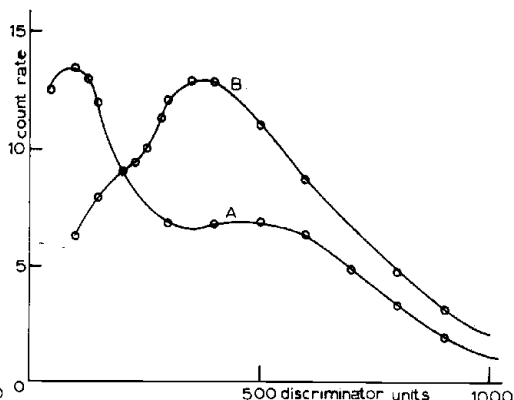


Fig. 8.

Fig. 7. Pulse height spectra for unquenched samples of ^{125}I in toluene/butyl-PBD in the absence (curve A) and presence (curve B) of 1 ml $\text{Sn}(\text{CH}_3)_4$.

Fig. 8. Pulse height spectra for quenched samples of ^{125}I in toluene/butyl-PBD in the absence (curve A) and presence (curve B) of 1 ml $\text{Sn}(\text{CH}_3)_4$.

From the reduction in counting efficiency on the γ -counter for tetramethyl tin (Fig. 5), it should be possible to predict the increase in counting efficiency on the β -counter. The fact that the observed change in count rate with increase in additive deviates from that predicted (Fig. 10) is explained by the fact that the predicted value

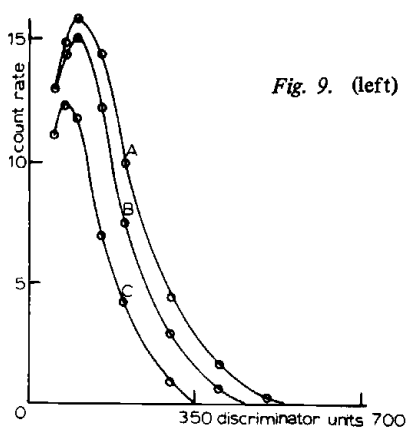


Fig. 9. (left)

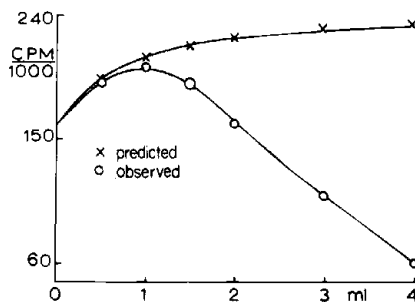


Fig. 10

Fig. 9. Pulse height spectra for samples of ^3H in toluene/butyl-PBD. (A) Unquenched sample. (B) Sample A + 1 ml of $\text{Sn}(\text{CH}_3)_4$. (C) Sample B + 0.45 ml of acetone.

Fig. 10. Plot of ^{125}I counting rate against volume of added $\text{Sn}(\text{CH}_3)_4$: O — observed curve for the liquid scintillation counter; X — curve predicted from γ -counting data assuming no impurity quench effect.

takes no account of the impurity quenching effect. Up to 1 ml of additive quenching is negligible, above this volume it becomes increasingly important. It would appear then that 1 ml is the optimum volume for tetramethyl tin.

Figure 11 compares the volume effect of both additives. Tetrabutyl tin has neither such a dramatic enhancement nor quenching effect as tetramethyl tin, although in terms of moles the differences are less pronounced (Fig. 12).

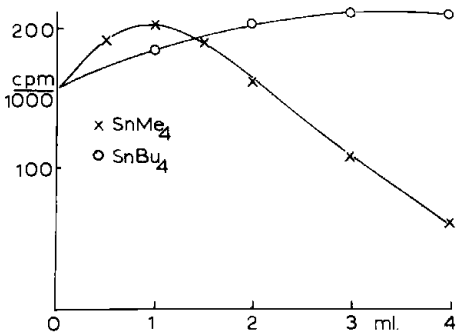


Fig. 11.

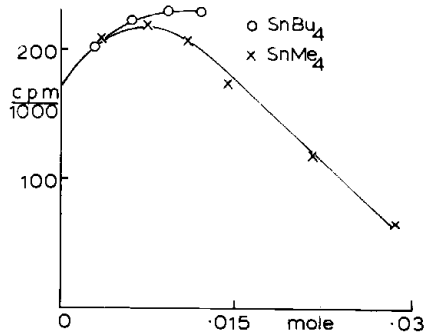


Fig. 12.

Fig. 11. Plot of ^{125}I counting rate against volume of added organotin compound.

Fig. 12. Plot of ^{125}I counting rate against moles of organotin compounds.

Figure 13 illustrates the volume effect of both additives on ^3H and ^{125}I samples both having an initial count rate of $150,000 \text{ counts min}^{-1}$. The optimum increase in count rate of ^{125}I requires 1 ml of tetramethyl tin and 2 ml of tetrabutyl tin. The same volumes result in ^3H count rates of $50,000 \text{ counts min}^{-1}$ and $90,000 \text{ counts min}^{-1}$ respectively. Bearing in mind that we wish to achieve the greatest possible relative change in counting efficiency, it is clear that 1 ml of tetramethyl tin represents the optimum volume of the more suitable reagent.

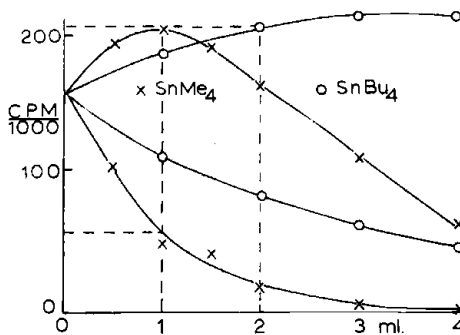


Fig. 13. Summary of the effects of added volumes of organotin compounds on the counting rates of ^3H and ^{125}I samples in toluene/butyl-PBD.

Figure 14 illustrates the four quench correction curves and the efficiencies of a typical sample. The effect of adding 1 ml of tetramethyl tin is to increase the ^{125}I efficiency from 68% to 85% and to reduce the ^3H efficiency from 31% to 24%. Typical results are shown in Table 3. The results are reasonable. They are better than those obtained using Method 2 and, although not as good as the best results obtained by Method 1, are vastly superior when Method 1 is subject to significant variations in γ counting efficiencies.

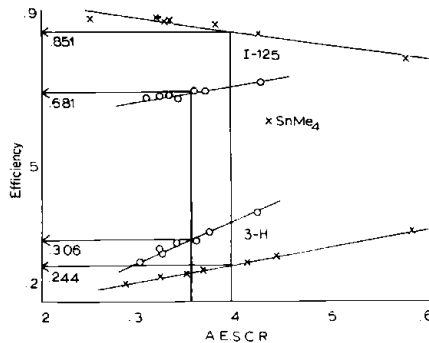


Fig. 14. Quench correction curves for ^3H and ^{125}I samples: O – in the absence of organotin compound; X – in the presence of 1 ml of $\text{Sn}(\text{CH}_3)_4$.

Table 3. Results obtained using a liquid scintillation counter and efficiency variation (Method 3).

Observed ^{125}I activity (dpm)	Recovery (%)	Attenuator setting	Observed ^3H activity (dpm)	Recovery (%)
17582	108.0	0.5	106512	97.2
17248	105.9		108057	98.6
17161	105.4		106971	97.6
17023	104.6	1.0	107154	97.9
17526	107.6		106635	96.8
17407	106.9		104792	95.7
16948	104.1	1.5	106286	97.0
17156	105.3		106470	97.2
15822	97.1		105188	96.0
16612	102.0	2.0	106599	102.0
16743	102.8		107480	98.1
16332	100.2		107724	98.3

EXPERIMENTAL

Sodium [^{125}I] iodide was supplied by the Radiochemical Centre, Amersham, England. [^{125}I] Iodobenzene was prepared from sodium [^{125}I] iodide and aniline by the method of diazotisation.⁴ Counting equipment used was a Packard Model 3314 liquid scintillation counter, a Philips PW4510 liquid scintillation analyser and a Packard Model 578 γ -counter.

CONCLUSIONS

Three methods for the assay of doubly labelled samples containing ^{125}I and ^3H have been investigated.

Method 1 under ideal conditions provides results of the greatest accuracy, but is sensitive to γ -photon absorption which cannot easily be monitored. Method 2 is both time-consuming and inaccurate and is therefore of little value. The results obtained by Method 3 are reasonably accurate. It is confidently expected that improved results will be obtained using this novel method when other additives have been investigated. The method described uses only one window of a liquid scintillation counter. It may well be that, although energy discrimination alone does not provide a reliable basis for an assay method, a double channel approach with two-way spillover will improve the results obtained from Method 3. This also requires to be investigated.

REFERENCES

1. W. R. Greig, in *Liquid Scintillation Counting*, Vol. 1 (Ed. A. Dyer), Heyden, London, 1970, p.120.
2. W. R. Greig, private communication.
3. J. Ashcroft, *Anal. Biochem.* 37, 268 (1970).
4. H. J. Lucas and E. R. Kennedy, *Org. Synth. Coll.* 2, 351 (1943).

DISCUSSION

V. Tarkkanen: Your problem seems to arise from the simultaneous quenching of ^{125}I and ^3H in a liquid scintillation counting spectrometer. Have you tried the mentioned simultaneous quenching of ^3H and efficiency improvement of ^{125}I by an inorganic density increaser like zinc chloride in water, combined with a colloidal scintillator? The organic tin compounds are expensive and toxic. Also the nature of biological aqueous samples requires a colloidal scintillator. We have found better results with an aqueous zinc chloride solution combined with a colloidal scintillator (1:1 water solution: Insta-Gel) than with organic tin derivatives.

K. L. Evans: Sodium iodide, for instance, gives a lower efficiency than the tin compounds. We have not tried zinc chloride merely because in this preliminary investigation we preferred a homogeneous counting sample. Indeed the cost of tetramethyl tin is considerable — about £1 per ml. Your proposal seems to be very interesting indeed.