

# Fundamental Approaches for the Assessment of Chemical and Colour Quenching in Backgrounds and Samples

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## INTRODUCTION

In this presentation two quite different aspects of quenching will be discussed. Both give rise in certain circumstances to considerable errors in the final calculation of disintegration rate, but nevertheless many workers are unaware of or ignore them. The topics are (1) the change in the background counting rate at various degrees of quenching, and (2) the very different shapes of pulse height spectra observed for colour and chemically quenched samples and the consequent problem of the assessment of quenching using the pulse height shift procedure.

## CHANGES IN BACKGROUND WITH QUENCHING

This subject is of special interest when low activity samples with various levels of quench are being counted or when the blank (as distinct from the background) from, for example, an enzyme assay is several times the unquenched background. If external standard values are being used to estimate quenching, then the subtraction of the corrected background is readily achieved, but the background counts depend on the fluor and quenching agent as well as the instrument.<sup>1</sup> Here it will be shown that it is possible to subtract the corrected background when the channels ratio procedure is employed to assess quenching. In this case the ratio itself is of course affected when dealing with samples having low activities of up to five to ten times the true background. A computer program will be described which can be used to calculate the appropriate background for not only single-label experiments but also for those involving double labels when the two ratio channels are set in the pulse height range for the higher energy isotope.

The problem which the program solves is illustrated in Table 1. It can be seen that not correcting for background or subtracting a constant background count

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gives rise to a gross error in the true channels ratio, which is of course used to assess the counting efficiency of the sample. The results in Table 2 show that

Table 1.

Using a constant background			
	Channel 1	Channel 2	Channels ratio
Background (c.p.m.)	30	20	
Gross (c.p.m.)	70	45	1.55
Sample (c.p.m.)	45	25	1.60
Using a quenched background			
	Channel 1	Channel 2	Channels ratio
Background (c.p.m.)	37	15	
Gross (c.p.m.)	70	45	1.55
Sample (c.p.m.)	33	30	1.10

Table 2.

Using a constant background			
	Channel 1	Channel 2	Channels ratio
Background (c.p.m.)	168	70	
Gross (c.p.m.)	205	135	1.52
Sample (c.p.m.)	37	65	0.57
Using correct quenched background			
	Channel 1	Channel 2	Channels ratio
Background (c.p.m.)	133	107	
Gross (c.p.m.)	205	135	1.52
Sample (c.p.m.)	72	28	2.57

similar errors are encountered when the background count is somewhat higher. This may be the case for an analytical procedure where the blank is high. Thus there is a quenching of the true background as well as in the blank. These may well be affected in different ways and it is clear that if any reliance is to be placed on the assessment of the efficiency using the channels ratio procedure, it is mandatory that the corrected background be introduced into the calculation.

Figure 1 shows the change in the counting rate for coloured and chemically quenched samples in three different energy channels. Figure 2 illustrates the effect when samples have a large 'background' made up of the true background plus the blank. These results were obtained by placing samples in vials which were specially selected for their similar backgrounds in a wide tritium window. Each vial was counted for 20 min during each of ten cycles and then vials were selected which had means within  $\pm 0.8$  c.p.m. of a running mean. Workers undertaking low activity measurements should make a similar selection of vials. A wide tritium window is a better and generally more stringent setting for double-isotope work with Hydrogen-3 and Carbon-14.

The computer program is written in Fortran Extended and runs on a CDC 6400 instrument. The code is structured so that the correct channels ratio is attained using an iterative procedure and depends on the input of data from a quenched set of radioactive standards and a set of backgrounds quenched in precisely the same fashion. Each set should have at least six standards. Figure 3 illustrates the flow chart for the program. The program is written to allow the user maximum

flexibility; thus on the first card the input format is punched and on the next one the number of standards and the channels to be used for the ratio. Default values of six standards and channels 2 divided by 3 are provided. The program then

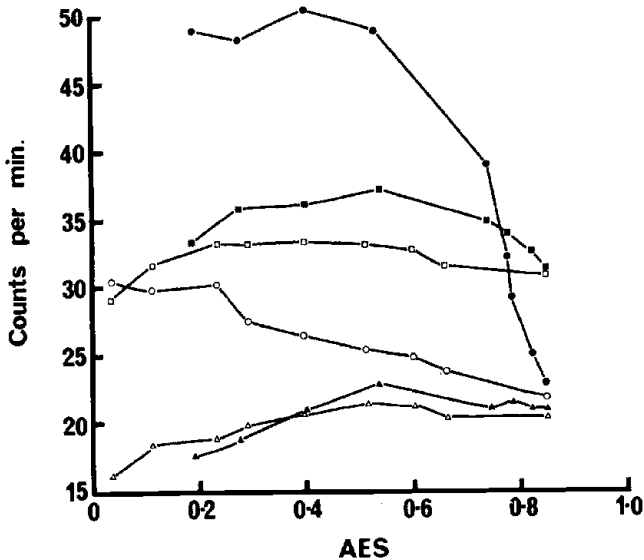


Fig. 1. Background counts per minute for colour and chemically quenched samples. Methyl orange in ethanol and carbon tetrachloride were the quenching agents. Readings were made on a Packard Tricarb 3375 operated at 20°C and fitted with EMI QB9635 photomultipliers. They are the means of ten 20-min counting periods for each sample. AES = automatic external standard ratio.

- Chemical quench. P.H.A. setting 20–1000 at 45% (wide tritium window)
- As above but colour quenched.
- Chemical quench. P.H.A. setting 50–1000 at 6% (20:1 Carbon-14 window)
- As above but colour quenched.
- △ Chemical quench. P.H.A. setting 130–1000 at 6% (Carbon-14 above Hydrogen-3 endpoint).
- ▲ As above but colour quenched.

calculates counts per minute for both sets of standards and ratios of the labelled standards, and the ratios are then associated with the identically quenched background for each channel. A sample is then read and the channels ratio, CR1, is obtained from it. If the sample is of medium to low activity, CR1 is probably not the correct value for assessing the counting efficiency. This value is tested as follows. It is used to obtain, by linear interpolation, backgrounds for each channel. If CR1 is larger than the biggest ratio the backgrounds appropriate to that ratio are used, and similarly for the lowest ratio. These backgrounds are then subtracted from the gross counting rate in each channel for the sample and a new ratio, CR2, is derived. If CR1 and CR2 differ by no more than 0.2%, then CR2 is used to obtain backgrounds and the result is printed prior to reading the next sample. However, if CR1 and CR2 are not that close, then the program sets CR1 equal to CR2 and the process starts again and is continued for up to 40 times, after which an informative message is printed. Under certain circumstances, CR1 and CR2 oscillate between the same two values and if this occurs a weighting

factor is included so that the new CR1 is not equal to CR2 but falls at a definite point in between the two values. This assumes that there is some validity in the values of both CR1 and CR2. In this way a proper convergence is attained. The program terminates with a blank card. The user is protected in the case of

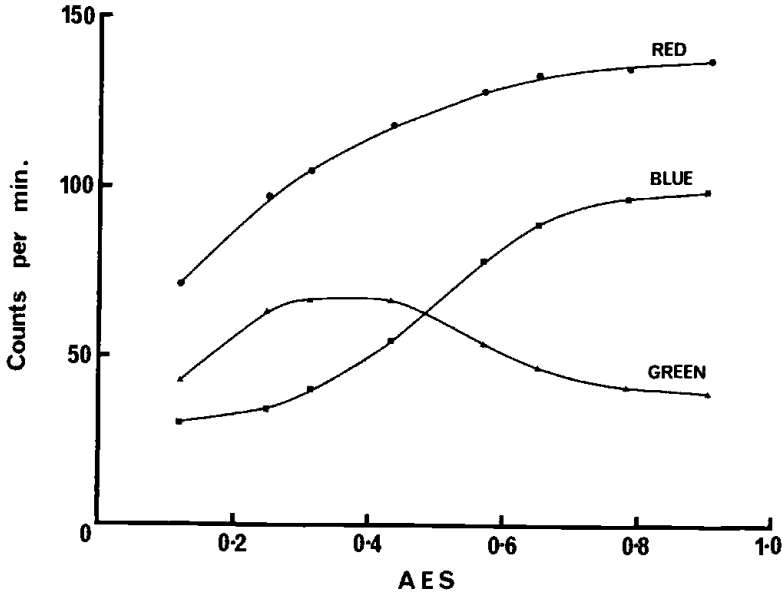


Fig. 2. Background plus Carbor-14 blank counts per minute. The quenching agent was carbon tetrachloride. Instrument as in Fig. 1.

RED P.H.A. setting 50-1000 at 6% (20:1 Carbon-14 window).

GREEN P.H.A. setting 50-150 at 6%.

BLUE P.H.A. setting 150-1000 at 6%

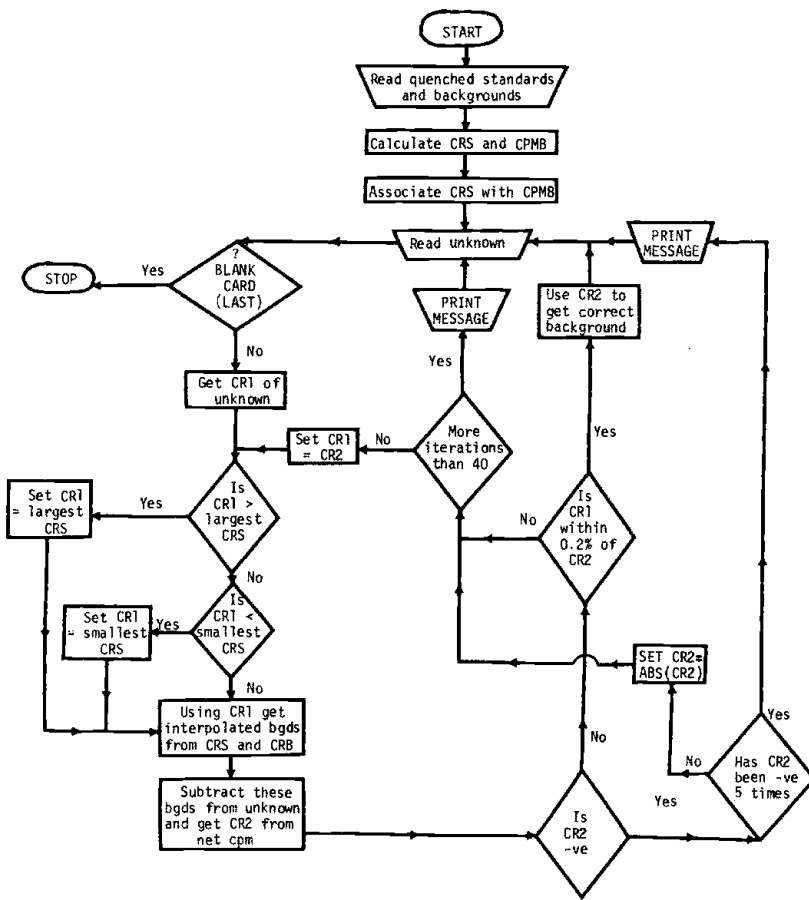
non-convergence and when samples have an apparently lower counts per minute than the most quenched standard. Thus, in the latter case, one of both channels giving rise to negative net counts per minute causes the program to reassign to that data positive values and iterations are allowed to continue for up to five occasions, after which time an informative message is printed and the next sample is read in.

The program works well in practice and if the user counts the samples to satisfactory statistical limits it usually gives a result for each sample rather than printing the default message. The program is readily adapted for double-label work when the two ratio channels are set above the endpoint of the lower energy isotope. However, the dynamic range for assessment of quenching by this procedure is somewhat limited<sup>2,3</sup> since as quenching increases the ratios usually either tend to a value of one or zero, depending on the way in which the discriminators are set. Recently Hansen and Carroll<sup>4</sup> extended the useful range of this procedure by setting the lower discriminators for the upper energy isotope below the endpoint for the lower energy isotope. The value of this procedure is shown in Fig. 4 but it means that the lower energy isotope increasingly contaminates the ratio channels as the discriminator is set lower and Carbon-14 is counted with better efficiency, thus allowing a bigger dynamic range for assessment of quenching. Hansen and Carroll obtain the correct ratio and hence efficiency by an iterative procedure. By employing their procedure

together with the program described here, it should be possible to obtain good assessments of quenched background thus:

1. set channels ratio CR3 = 100.0;
2. use the spillover procedure of Hansen and Carroll<sup>4</sup> to obtain a channels ratio CR4;
3. is CR3 within 0.2% of CR4? If yes, use CR4 to get backgrounds and then exit. If no, go to 4;
4. more than 50 iterations? If yes, print default message and exit. Otherwise, go to 5;
5. using CR4, obtain new background and thus a new ratio CR3. Go to 2.

Work is continuing to complete this part of the program and also to assess whether the use of differences in successive background determinations makes a better and quicker termination of iterations than the difference between CR1 and CR2 and also CR3 and CR4.



CRS = Channels ratio of standards  
 CPMB = CPM's of background standards  
 CR1 and CR2 - Channels ratio calculated by programme

Fig. 3. Flow diagram for the computer program which calculates the correct value for the quenched background when using the channel ratio procedure.

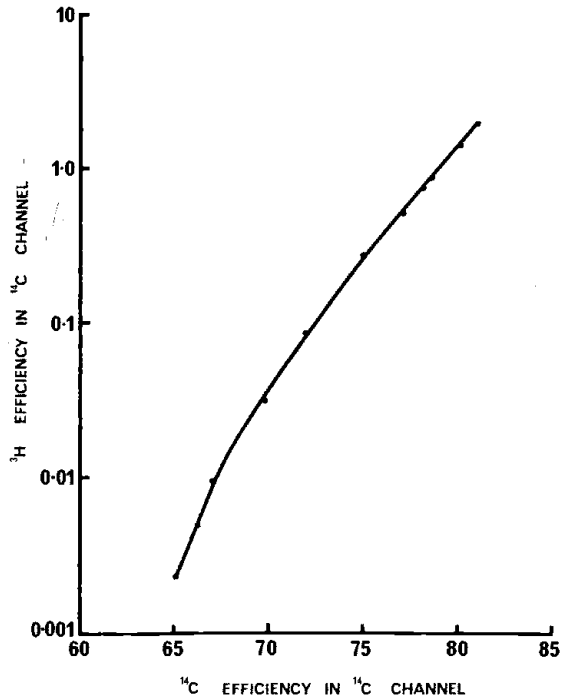


Fig.4. Spillover of Hydrogen-3 into the Carbon-14 channel as a function of Carbon-14 efficiency. Data were collected for an oxygen quenched sample using the instrument mentioned in the legend to Fig.1. The lower discriminator of the carbon channel was set to give around 5% Hydrogen-3 efficiency and then gradually raised. This illustrates the value of setting this discriminator some way into the tritium spectrum, as there is considerable gain in Carbon-14 efficiency (and quenching range accessible by channels ratio) for only a marginal change in the spillover of Hydrogen-3.

Some typical results obtained using the program are presented in Table 3.

Table 3. An example of the results produced by the program.

Channel	Gross c.p.m.	Ratio 1/2	Actual net c.p.m.	Ratio 1/2	Calculated net c.p.m.	Ratio 1/2
1	78.7		56.0		54.6	
		1.81		2.58		2.59
2	43.5		21.7		21.1	
3	20.2		6.4		5.7	

#### A NEW APPROACH TO THE PROBLEM OF CHEMICAL AND COLOUR QUENCHING

The pulse height spectra for chemically and colour quenched samples of medium-high energy  $\beta$ -emitters are different in shape. This difference is significant with Carbon-14 (see Fig. 5) but is barely discernible with the low energy emitter tritium, since it is closely associated with the number of photons produced by the  $\beta$ -particles. Thus, for a mean  $\beta$ -energy (50 keV) from Carbon-14 about 800 photons

are produced,<sup>5</sup> while for Hydrogen-3 (mean 5 keV) the value is much lower, around 50. Since about one-fifth of this number finally results in the production of a photoelectron, it follows that for tritium a much smaller number of photoelectrons is involved; thus the difference between the effects of the two types of quenching is small. However, for Carbon-14 when larger numbers of photons are involved, there is more scope for statistical variation at all stages in the process and the difference in spectra occurs because of this.

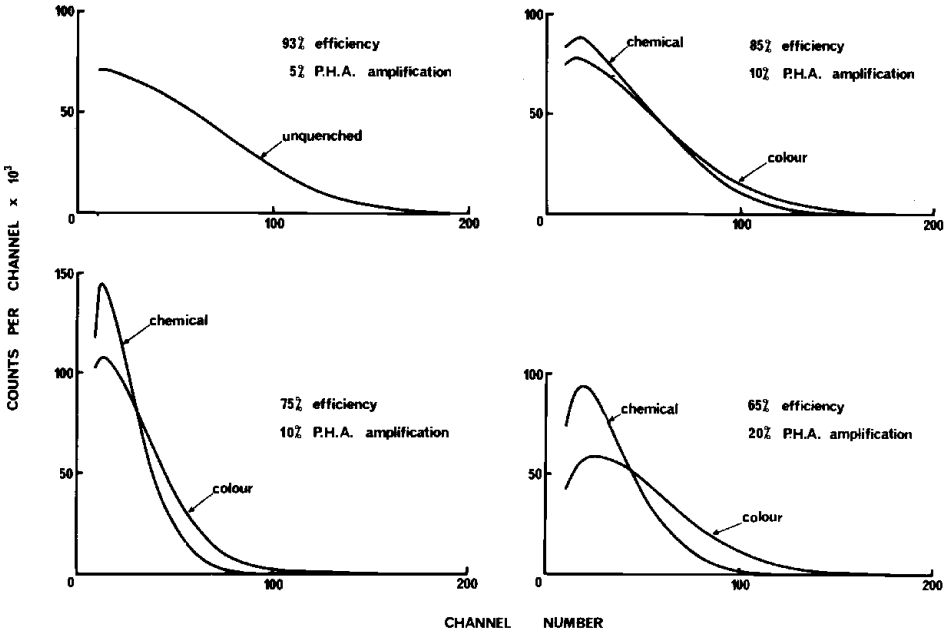


Fig. 5. Pulse height spectra for quenched Carbon-14 samples. Carbon tetrachloride and methyl orange were the chemical and colour quenching agents respectively. The 'unquenched' sample was air saturated. Quoted efficiencies were obtained in coincidence at integral settings on a Packard Tricarb 3390-544 fitted with RCA 4501 V4 bialkali photomultipliers and operated at  $20 \pm 0.5^\circ\text{C}$  (3 to  $\infty$  at 100% amplification). Pulse height spectra were obtained using a Packard Spectrazoom 200 channel multichannel analyzer connected by a coaxial cable via a 1K resistor (for pulse stretching) to the test point at the rear of the RED pulse height analyzer. Coincidence was established by a pulse from the RED ratemeter socket. The spectra were conveniently fitted into the 200 channel analyzer by varying the pulse height analyzer gain (P.H.A.).

It is worth while to explain briefly the fundamental differences between colour and chemical quenching. Chemical quenching results in a diminution of the photon output from a scintillation and the effect is quite independent of the position in the vial where the event occurs. However, colour quenching involves the absorption of emitted photons by the sample itself. Thus, as the path length taken by a photon increases, the likelihood of it being absorbed increases exponentially. Taking a hypothetical  $\beta$ -particle producing a scintillation at the centre of the vial and the photons (of similar wavelength) being emitted in the plane of the vial base,

then the probability that each photon will reach one photomultiplier is about the same. However, for a scintillation occurring very close to one photomultiplier, then photons emitted in its direction will have a much higher probability of reaching it than photons having to traverse the diameter of the vial to the other photomultiplier.

Thus, for example, given a total of 1000 photons reaching the photomultipliers, the pulse produced at the nearer photomultiplier may be the result of 900 of these and consequently a pulse height ratio of 9:1 is recorded for the photomultipliers. For the scintillation occurring in the middle of the vial, the ratio would be about 5:5. This, of course, is an over-simplified way of considering the process since photons are not of constant wavelength, do not travel in one plane and do not divide themselves equally to the left and right directions. It does, however, illustrate the point that the pulse height spectrum for colour quenched samples is the result of a highly probabilistic set of factors. It is therefore apparent that in attempting to apply the channels ratio procedure for  $\beta$ -emitting isotopes which are more energetic than tritium, it is necessary to use two standard curves, one for colour and the other for chemical quenching.

Thus it is not good practice to use the ratio procedure to assess the chemical and colour quenching in the same sample. Very recently Ediss *et al.*<sup>6</sup> have shown that reasonably effective corrections can be applied if the smaller of the two pulses is used to produce the spectrum for analysis. This is known as lesser pulse height analysis. The present work has had as its long-term aim the analysis of the shape of the pulse height spectrum so that a quantitative assessment of both colour and chemical quenching can be made in the same sample. To do this a mathematical model is being developed to study the factors that are important in influencing the shape of the pulse height spectrum starting with the  $\beta$ -particle itself. A much more detailed account of the present model was presented by Malcolm and Stanley at a recent symposium.<sup>7</sup> Only a brief synopsis will be given here.

Monte Carlo techniques are used in simulating various stages of the liquid scintillation counting process. As a result of this, the amount of central processor time (CP) needed for each scintillation for Carbon-14 is considerable, i. e. approximately 1 s on a CDC 6400 computer. Thus, to obtain a reasonable pulse height spectrum, at least 10,000 s (nearly 3 h) of CP time is required. The program, written in Fortran Extended, requires less than  $40^8$  K core.

The simulation is initiated by the computer generating a hypothetical  $\beta$ -particle in a random three-dimensional position within the vial. The  $\beta$ -particle is then randomly assigned an energy according to the Fermi probability function for Carbon-14. Currently 13 photons are then assigned per keV of the  $\beta$ -particle. Earlier in this symposium Dr. Horrocks showed that the number of photons produced per keV of energy was energy dependent below 300 keV and so it will now be necessary to modify the model to include this correction. If chemical quenching is to be simulated, a fixed fraction of photons for each scintillation is discarded at this stage. The photons produced are then randomly assigned different wavelengths according to the fluorescence spectrum of PPO in toluene and are emitted in random directions throughout the vial. From the measured absorption spectrum of colour quenched samples, the photons of appropriate wavelength are then either absorbed or not, prior to reaching the vial wall. This is accomplished using an exponential probability function and the random number generator. The next stage in the simulation is to determine the angle of incidence of the photon at the vial-air interface. If it is greater than the critical angle, then the photon will be totally reflected internally and thus lost.

The current version of the model produces a value of approximately 50% loss of photons by this process, the refractive index of toluene and the glass used for making vials each being approximately 1.50. Evidence for the considerable loss of light in the vial walls by the process of internal reflection is given in Fig. 6. The sample elevator apparently does not reflect much of this light towards the photomultipliers, since covering the elevator with black paper diminishes the counting efficiency by only around 1%.

It is presently assumed that the detector chamber is perfectly reflective and there are no transmission losses in air. Moreover, photons are not reflected back into the vial but go directly to the right-hand photomultiplier if their initial path is to the right of the line perpendicular to the photomultipliers or to the left-hand one if the initial path is to the left. Thus photons arrive at each photomultiplier and the photocathode is then modelled by taking account of its quantum efficiency for different photon wavelengths. Again using a probability function and the random number generator, a photon of a given wavelength either produces a photoelectron or does not. Coincidence is modelled simply by requiring that at least one photoelectron has been generated at each photocathode during the simulation of a  $\beta$ -event and scintillation. Summation of the coincident pulses simulated the summation amplifier.

While work is still progressing on this model, it currently produces spectra for coloured and chemically quenched Carbon-14 samples which are qualitatively

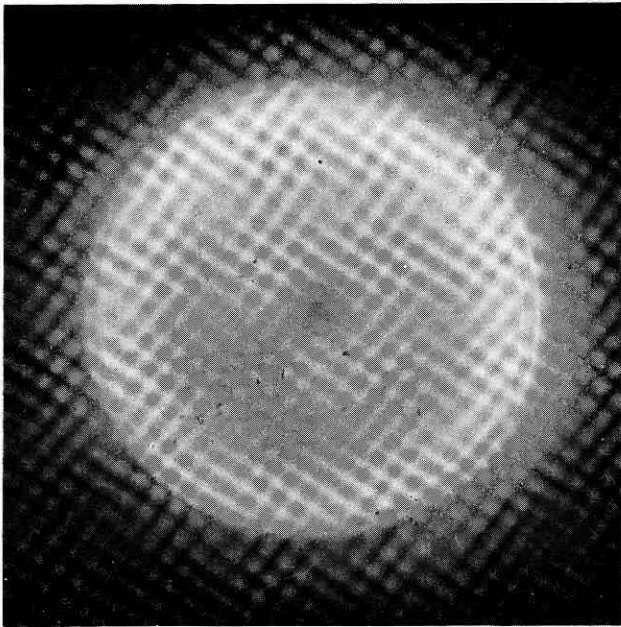


Fig. 6. A photograph of the bottom of a scintillation vial filled with a Carbon-14 sample, taken by its own light. Notice the lighter annulus at the rim of the vial. This is thought to be caused as follows. A photon which gets totally internally reflected in the upper vial walls by virtue of their circular symmetry will occasionally be reflected towards the base. Here there is an abrupt change in symmetry, especially near the rim, and thus more light escapes at this point.

similar to those observed in practice, and the integral counting efficiencies, down to 65%, are within a few per cent. It is of interest that satisfactory results for tritium are obtained with about 11 photons per keV instead of the 13 for Carbon-14. The reason for this has been unclear but the values given by Dr. Horrocks for the scintillation efficiencies of 0.044 and 0.052 may well explain this result. Further additions to the model will include information on the optics of both the detector chamber and the vial and the perturbations of pulses known to occur in the photomultiplier.<sup>8</sup>

#### ACKNOWLEDGEMENTS

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#### DISCUSSION

B. E. Gordon: How do you square the 50% total internal reflection loss with only 5% increase in efficiency of Hydrogen-3 counting?

P. E. Stanley: The important thing is to recall that all you need to detect an event is a coincidental pulse from each photomultiplier. If more photons are collected at the photocathode then this increases the pulse height but not the efficiency. What you are really increasing when using sand blasted vials is the number of few photon events which enable coincidence since the photons are not so readily totally internally reflected.

B. W. Fox: Was the photograph of light emission which you showed done with an optically flat vial base – it looked remarkably like the unprecise moulding on our own vials?

P. E. Stanley: Yes, the variation may be due to this source. What I attempted to demonstrate was that the lower corners of the vial represent a non-symmetrical area and photons being totally internally reflected on the vertical vial walls would stand a better chance of leaving the vial at this point.

**B.W. Fox:** Has anyone considered vertical moulding on vials, much like some modern lighting techniques, to increase the light output from the vial?

**P.E. Stanley:** No, except for Dr. Gordon's work in attempting to define the light output by various surfaces.

Author's note added in proof

The efficiencies for Carbon-14 counting mentioned in Fig. 5 are probably about 3 percent too high due to what is thought to be after-pulsing in the photomultipliers. The shapes of the spectra are unchanged since counts in the first few channels, where after-pulses would be apparent, are not included. Normal pulse height analyser settings for Carbon-14 would, to a large extent, eliminate after-pulses which have only a small pulse height yet may be resolved by the pulse height analyser from the main scintillation pulse.