Liquid Scintillation Counting with a Single Photomultiplier Tube

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Abstract

The channels ratio method of quench correction has been examined and found to be applicable when a single photomultiplier is used for liquid scintillation counting of carbon-14 and tritium. The relatively simple equipment is also suitable for assay of doubly labelled compounds provided that quenching is not important.

Introduction.

Liquid scintillation counting of isotopes which emit weak β-particles is now a well established technique with very wide applications. The advantages, disadvantages and utility of liquid scintillation counters, compared with other instruments, have been adequately summarised elsewhere [1-4] particularly with reference to sophisticated spectrometers using two photomultiplier tubes and several channels of pulse height analysis. The results reported in this paper refer to work with a single photomultiplier coupled to a simple pulse height analyser and are logical extensions of work using the more complex coincidence counters.

The advantages of using a single photomultiplier relate mostly to the simplicity of the associated electronic equipment with consequent financial benefits. Such considerations are still relevant in many laboratories where the utmost sensitivity and precision are not always required and where the number of samples to be counted are relatively few.

Experimental.

(i) *Apparatus.* The counting equipment consisted of an Ekco liquid scintillation counter (type N664A) cooled to —10° C in a Frigidaire deep-freeze

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cabinet (type JCR35) and connected to an Ekco scaler (type N610A). The scaler incorporates a simple pulse height analyser (p.h.a.) with variable upper and lower discriminators. A second fast scaler was one constructed in the electronics workshop of this College but any fast scaler would have sufficed. The counting bottles were as supplied by the manufacturers with a white reflecting paint on the upper surfaces so that changes in sample volume did not affect the overall count rate.

(ii) *Materials.* "Analar" toluene was dried and redistilled. Ethyl alcohol, acetone and carbon tetrachloride were "Analar" grade and were used without further purification. Picramide (2,4,6-trinitro-aniline) was a pure sample used in other research. PPO, POPOP and IM hyamine hydroxide in methanol were as supplied by Nuclear Enterprises (G.B.)Ltd. Toluene-^{14}C and toluene-^{3}H were supplied by the Radiochemical Centre, Amersham, and were suitably diluted with pure inactive toluene. Phenyl-^{3}H-*sec*-butyl-^{35}S$_1$-disulphide was a sample described elsewhere [5]. Activities were standardized against carbon-14 and tritium labelled hexadecane standards supplied by the Radiochemical Centre.

(iii) *Procedure.* 10 ml of a phosphor solution consisting of PPO (0.3%) and POPOP (0.03%) in toluene were pipetted into a counting bottle. The radioactive substance was then added via an "Agla" micrometer syringe (Burroughs Wellcome & Co. Ltd.) and the bottle sealed and counted to give an "unquenched" count-rate. Subsequently the bottle was warmed to room temperature, opened and a pre-determined amount of quenching agent rapidly added via a second "Agla" syringe. The count rate of the quenched sample was then determined.

All the above operations were conducted in a room dimly lit by a tungsten filament lamp so that complications due to photo-luminescence were avoided. Counting bottles were placed in shallow trays of silicone oil (MS 200/2 cs) in the deep-freeze cabinet for at least 10 minutes to remove dust particles and to attain temperature equilibrium before transfer to the counter.

RESULTS AND DISCUSSION.

(i) *Counting Efficiency and quench corrections.* The occurrence of quenching of the light pulses in a liquid phosphor is one of the major problems associated with liquid scintillation counting. Quenching can be due to the dissolved sample, adventitious impurities, secondary (blending) solvents, excessive dilution or poor optical coupling between sample bottle and photomultiplier, and in our experience is both variable and unpredictable. In the past we have routinely counted samples, added an internal standard and re-counted to obtain a value for the counting efficiency of every sample.

An alternative technique which does not involve removal of the sample from the counter was first discussed by Davidson [6] and subsequently developed

by Baillie [7] and Bruno and Christian [8]. The method utilises the fact that
quenching of the light output from all energies of particles causes a general
shift of the pulse height spectrum toward lower energies. Thus, inspection of
separate sections of the pulse height spectrum reveals any variations in the
extent of quenching. This technique has now been applied using a single photo-
multiplier counter and shown to be of similar precision to the internal standard
technique.

Figure 1 shows pulse height spectra for unquenched and quenched car-
bon-14 samples and Figure 2 shows similar curves plotted for tritium. These
curves were obtained by scanning pulse heights (in volts) from 5 to 50 volts
using a 2 volt window on the pulse height analyser. The lower limit was set
by the electronic equipment which would not accept pulses of less than 5 volts
and the upper limit was chosen arbitrarily just below the amplifier cut off
of about 60 volts. The high voltage to the photomultiplier (900 V. for ^{14}C;
1,000 V. for tritium) and the gain of the amplifier ($\times$ 1,000) were so adjusted
that a negligible number of pulses exceeded 50 volts for unquenched samples
(see Figs. 1 and 2). It was shown that these counting conditions were near to
the statistical optimum condition where [sample count rate]^2/[background
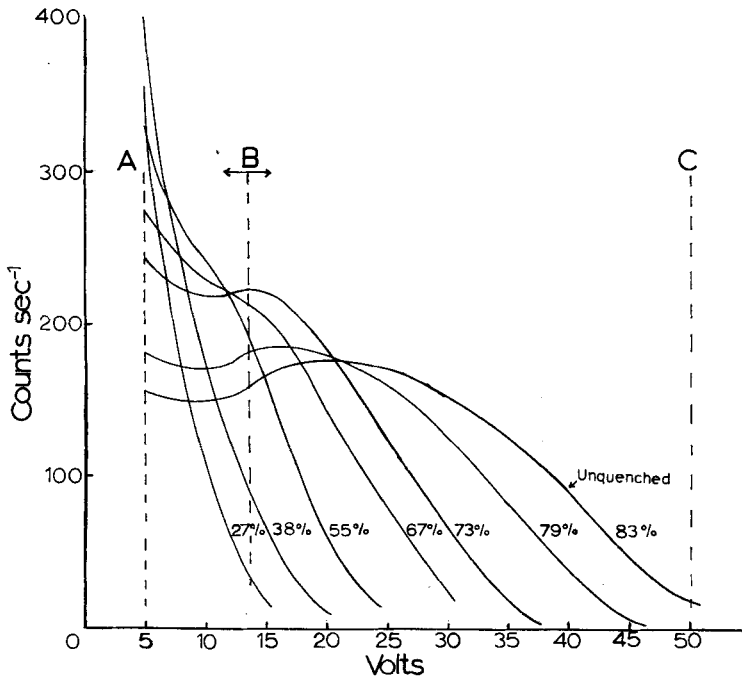count rate] was a maximum.

FIG. 1. Pulse height spectra of variously quenched samples of carbon-14. All the samples
contained the same amount of activity and counting efficiencies are as shown.
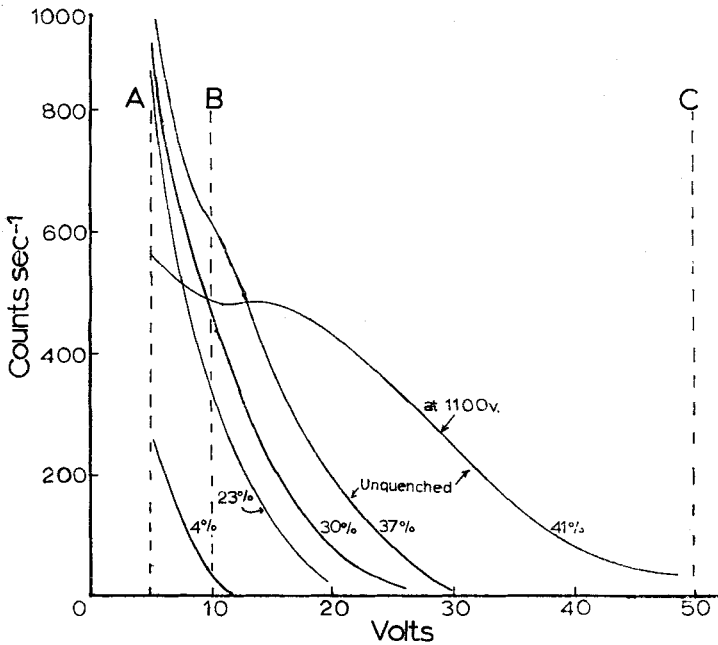
FIG. 2. Pulse height spectra of variously quenched samples of tritium. All the samples contained the same amount of activity and counting efficiencies are as shown. The spectrum on the far right was obtained by increasing the high voltage to 1,100 volts but the concomitant increase in background was unacceptable.

Referring to Figures 1 and 2, the shift to lower energies with quenching is clearly demonstrated and it follows that comparison of count-rates from two sections of the spectrum should give a measure of the extent of quenching. Baillie [7] and Bruno and Christian [8] plotted the ratio of counts in two channels (BC/AC, Figs. 1 and 2) against counter efficiency for the channel AC Using only a unmodified Ekco scaler type N610A the ratio BC/AB was most readily obtained and it was shown that this ratio was acceptable. The sample was first counted in the normal manner in the wide channel AC (i.e. with the "gate" off). The gate was then switched on and pulses in the AB channel recorded on the scaler. Pulses in the BC channel may be recorded simultaneously on the six decade timer used now as a second scaler. This is convenient because with the auto-stop on this second scaler set to 10,000 counts, the final reading on the first scaler gives the ratio AB/BC directly. There was a serious limitation in acceptable count rates, however, due to the high input resolution time of 250 μsec in the second scaler.

A more satisfactory solution was to use the timer for its intended purpose and to record BC pulses on an external slave fast scaler. When this arrangement was used it was only necessary to count once with the gate switched on

since the ratio BC/AB was obtained and the total count rate computed from
the sum of the total counts in the two channels divided by the time. Effi-
ciencies were then calculated using the known amounts of activity originally
introduced into the counting pots.

Figures 3 and 4 give the results obtained in the manner described above
for carbon-14 and tritium respectively. The positions of discriminators A
and B were selected rather arbitrarily as suggested by Baillie [7] but no syste-
matic investigation of the effects of variations in their settings was under-
taken. Such curves as shown in Figures 3 and 4 are acceptable over quite a
wide range of efficiencies for most chemical quenchers. It was also shown
that, for carbon-14, decreasing the high voltage on the photomultiplier had
exactly the same effect as quenching (Fig. 5). This important observation meant
that once a quench correction curve had been established there was no need
of exceptional care in setting up the high-voltage on different occasions. On
the other hand, coloured carbon-14 samples required a separate quench cor-
rection curve at lower efficiencies (see Fig. 5), though tritium samples did not.
These observations are in accord with the work of Baillie [7].

Using the correction curves to determine count-rates of quenched samples
it was possible to demonstrate that when reasonable amounts of activity
were present, the reproducibility of ±6% for carbon-14 was not quite as good
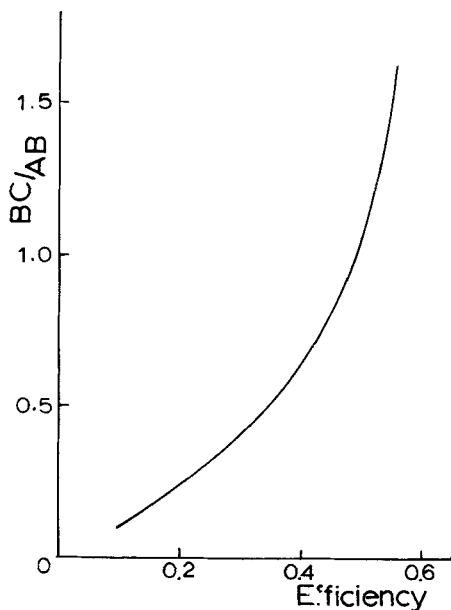as normally obtained when internal standards were used (±2 to 3%). The

FIG. 3. Quench correction curve for carbon-14 using the ratio BC/AB.
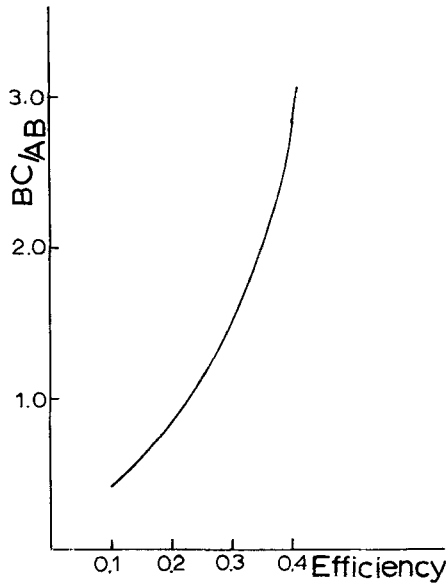
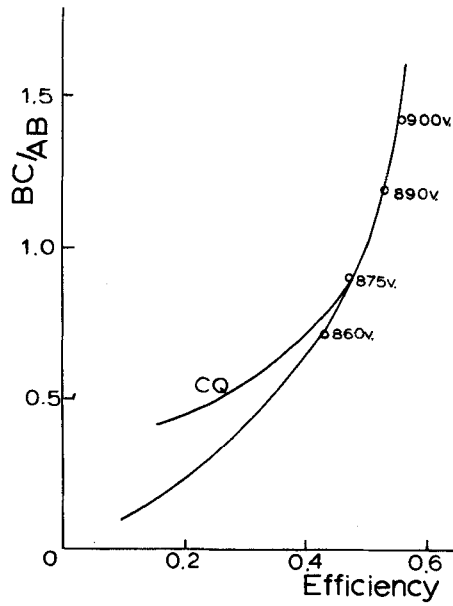FIG. 4. Quench correction curve for tritium using the ratio BC/AB.

FIG. 5. Effect of colour quenching (CQ) and variation in high voltage on the quench correction curve for carbon-14.

results for tritium, however, were comparable with the internal standard technique (Table I).

At lower activities, where background became important, there were indications that the reproducibility was much poorer. This was not thoroughly investigated since the long counting times involved and the need for precisely quenched background pots nullify any advantages the method has over the internal standard technique.

Inspection of Fig. 3 and Table I reveals that under the particular conditions used, the maximum counting efficiency for carbon-14 was slightly less than 60%. Further, Bush has investigated the channels ratio method of measuring counting efficiencies in considerable detail [9]. She pointed out that although the ratio BC/AB was best for tritium, a more satisfactory ratio for carbon-14 was AB/AC since the counting statistics were much improved at lower efficiencies. Consequently we investigated this procedure for the single

TABLE I. Results of activity measurements on quenched samples using the channels ratio BC/AB.

Isotope	Quench agent	Observed count rate (c/sec)	Efficiency	Corrected count rate (c/sec)
Carbon-14	—	2,703	.545	4,960
Carbon-14	Acetone	2,405	.500	4,810
Carbon-14	Acetone	1,039	.190	5,468
Carbon-14	Acetone	550	.105	5,238
Carbon-14	Ethanol	1,913	.400	4,783
Carbon-14	Ethanol	1,639	.341	4,806
Carbon-14	Ethanol	599	.137	4,372
Carbon-14	Carbon tetrachloride	2,078	.445	4,670
Carbon-14	Carbon tetrachloride	1,591	.338	4,707
Carbon-14	Carbon tetrachloride	1,193	.243	4,909
Carbon-14	Picramide	1,495	.308	4,854
Carbon-14	Picramide	790	.170	4,647
		Mean Value = 4,852 ± 5.8% [a]		
Tritium	—	663	.405	1,637
Tritium	Ethanol	587	.356	1,649
Tritium	Ethanol	396	.240	1,650
Tritium	Ethanol	172	.112	1,536
Tritium	Carbon tetrachloride	475	.287	1,655
Tritium	Carbon tetrachloride	355	.215	1,651
Tritium	Carbon tetrachloride	211	.128	1,648
Tritium	Picramide	535	.337	1,588
Tritium	Picramide	268	.174	1,540
		Mean Value = 1,617 ± 3.0% [a]		

[a] Standard deviation $(\sqrt{\Sigma\delta^2/n-1})$.

photomultiplier. The ratio could be obtained by counting the sample twice, first with the gate on (AB) and then with the gate switched off (AC). However, if as in the present case, a second scaler was available, the ratio could be obtained in one counting operation provided that the pulse height analyser was modified slightly. The modification involved insertion of a cathode follower buffer stage in the output of the upper discriminator followed by a simple pulse amplifier to feed the external scaler. In this way with the gate switched on, AB was obtained on the N610A scaler and AC on the external slave scaler.

Plots of the ratio AB/AC were prepared for carbon-14 and the effects of varying the various parameters were studied. Thus, Figure 6 shows a series of curves prepared with lower discriminator A constant at 5 volts and upper discriminator B set at various levels. If only slight quenching was expected in a series of experiments then curve (1) would give the greatest sensitivity. On the other hand if quenching was liable to vary widely then curve (4) which is linear over the entire range of efficiencies plotted (0.8-0.15) would be the obvious choice.

If the lower discriminator A is set at 10 volts then there is an improvement of background count rate from ~13 c/sec to ~3 c/sec for the wide channel AC. Thus, similar curves were prepared for A = 10 volts (Fig. 7) which showed the same features as those for A = 5 volts except that overall counting efficiencies were lower and the curves were slightly steeper. There seemed to be
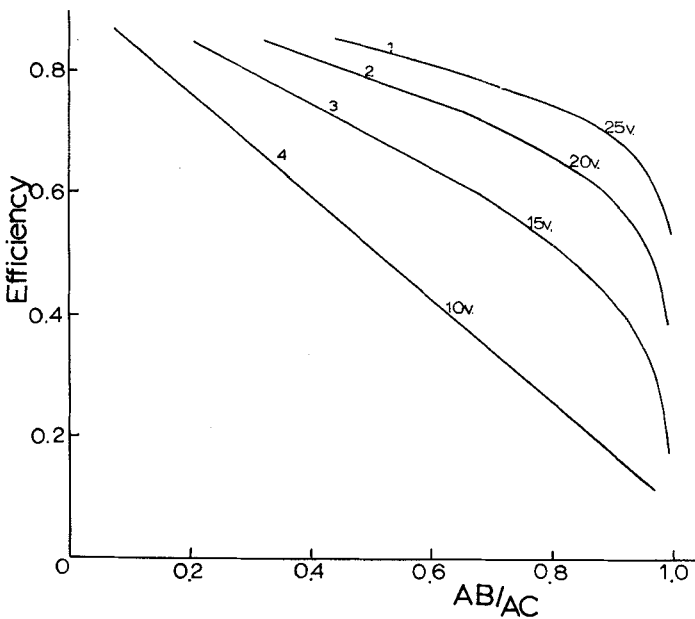

FIG. 6. Quench correction curves for carbon-14 using the ratio AB/AC. Lower discriminator voltage 5 volts, upper discriminator voltages as shown against each curve.
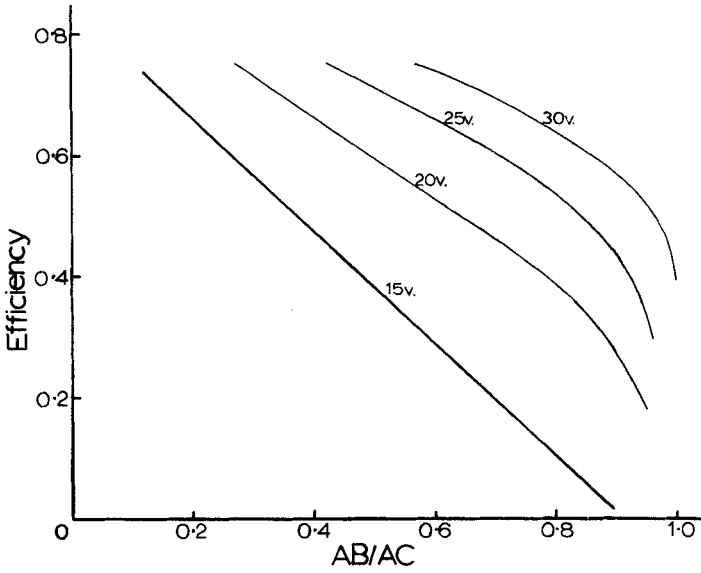
FIG. 7. Quench correction curves for carbon-14 using the ratio AB/AC. Lower discriminator voltage 10 volts, upper discriminator voltages as shown against each curve.
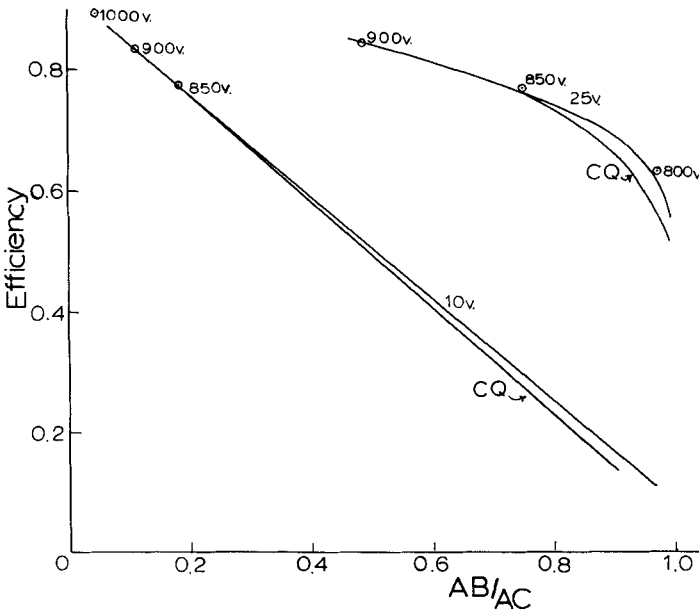
FIG. 8. Effect of colour quenching (CQ) and variations in high voltage on the quench correction curve for carbon-14.
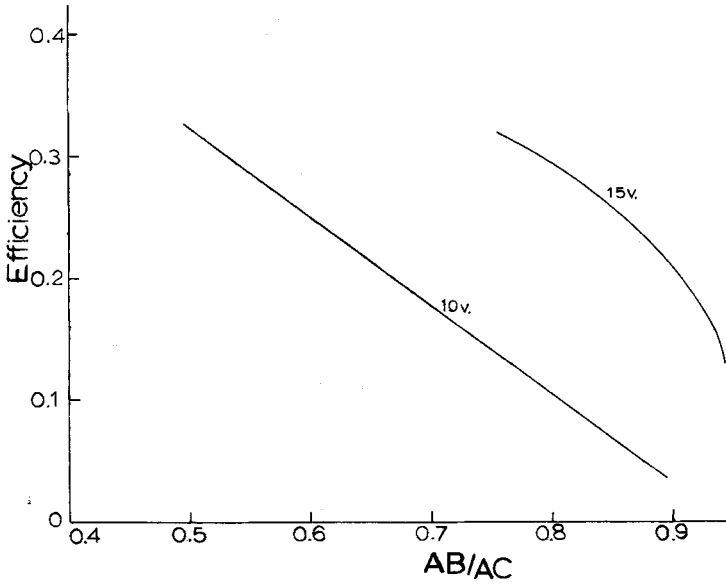
FIG. 9. Quench correction curves for tritium using the ratio AB/AC. Lower discriminator voltage 5 volts, upper discriminator voltages as shown.
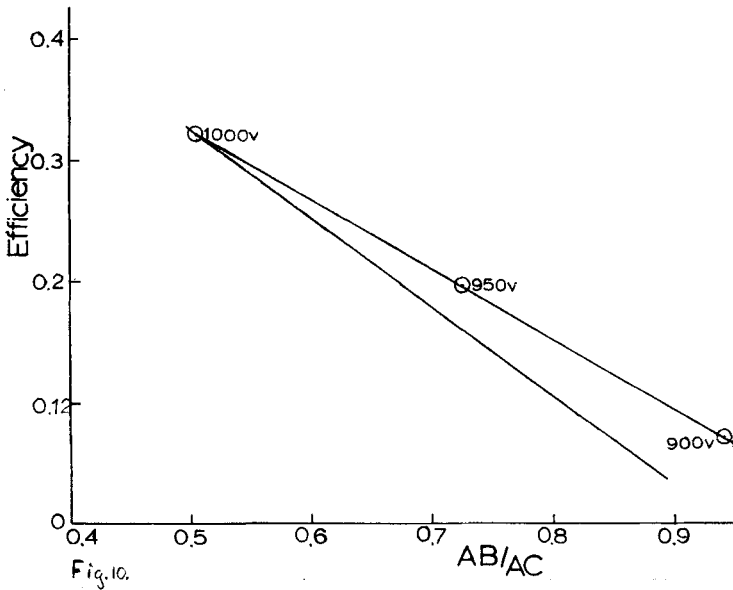

FIG. 10. Effect of high voltage changes on the quench correction curve for tritium.

no real advantage in using the higher setting for A since when sample count rates approach background levels the internal standard technique was preferred.

Further experiments showed that variations in high voltage both above and below the normal value gave points which fell on the curves just as described in the earlier case. Also, as before, coloured samples caused points to deviate from the normal curve but the deviations were not so marked, particularly on the linear correction curve (4) (Fig. 8).

Because of the modifications to the equipment it was decided to examine the effect of quenching on the ratio AB/AC for tritium. Notwithstanding Bush's preference for the ratio BC/AB it was found that for the same discriminator settings (A = 5 volts, B = 10 volts) the plot of AB/AC against efficiency was more linear in this case (Fig. 9) and might be more acceptable for some purposes despite the slightly reduced precision ($\pm 5\%$, Table II) and the fact that the change in ratio between the two extremes was not so great. Variation of the discriminator settings had much the same effect as in the carbon-14 case but changes of high voltage produced points which deviated from the calibration curve (see Fig. 10). The effect was not very marked for small changes in voltage but a drop of 50 volts would lead to an error of about 10% in the value for the counting efficiency. It was thought desirable in this
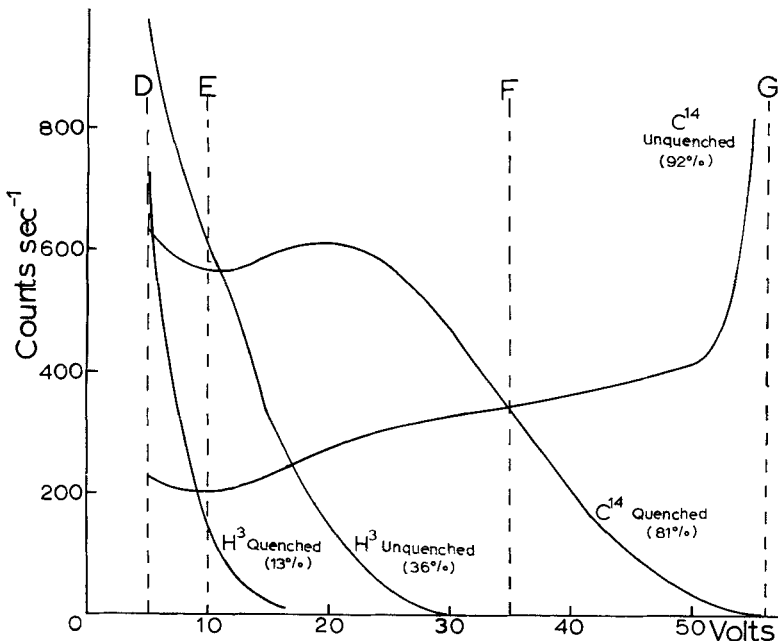

FIG. 11. Pulse height spectra for equivalent amounts of carbon-14 and tritium at the high voltage used for tritium counting. Counting efficiencies as shown, though the quenched samples have the same quantity of quenching agent added to each.

TABLE II. Results of activity measurements on quenched samples using the channels ratio AB/AC.

Isotope	Quench agent	Observed count rate (c/sec)	Efficiency	Corrected count rate (c/sec)
Carbon-14	—	2,606	.828	3,147
Carbon-14	Ethanol	2,394	.760	3,150
Carbon-14	Ethanol	1,717	.561	3,061
Carbon-14	Acetone	2,509	.798	3,144
Carbon-14	Acetone	2,227	.710	3,137
Carbon-14	Acetone	1,711	.536	3,192
Carbon-14	Acetone	858	.262	3,275
Carbon-14	Carbon tetrachloride	2,243	.717	3,128
Carbon-14	Carbon tetrachloride	552	.167	3,305
Carbon-14	⎫	2,187	.697	3,138
Carbon-14	Hyamine hydroxide ⎪	1,785	.570	3,132
Carbon-14	solution of a snail ⎨	1,020	.322	3,168
Carbon-14	⎭	602	.220	2,736
Carbon-14	Picramide	1,919	.620	3,095
Carbon-14	Picramide	1,415	.450	3,144
Carbon-14	Picramide	620	.207	2,995
			Mean Value = 3,122 ± 4.0% [a]	
Tritium	—	3,702	.310	11,942
Tritium	Ethanol	3,512	.307	11,439
Tritium	Ethanol	2,642	.226	11,690
Tritium	Acetone	2,728	.232	11,759
Tritium	Acetone	1,913	.155	12,342
Tritium	Acetone	868	.068	12,765
Tritium	Picramide	2,765	.238	11,618
Tritium	Picramide	1,566	.138	11,348
Tritium	Picramide	665	.062	10,726
			Mean Value = 11,737 ± 5.0% [a]	

[a] Standard deviation $(\sqrt{\Sigma \delta^2 / n - 1})$.

case to have a routine check for the quench curve which could readily be obtained with two or three sealed, quenched standards. No separate curve was required for colour quenched samples. The results of measurements with various quench agents using the linear correction curves are given in Table II where it can be seen that the precision for carbon-14 ($\pm 4\%$) is now nearly as good as with the internal standard technique.

(ii) *Double label experiments.* The problem of estimating tritium in the presence of carbon-14 or sulphur-35 has been investigated by several workers [10-13]. Figure 11 shows that, with the equipment set up for tritium counting, unquenched samples have pulse height spectra which are sufficiently different

to permit a reasonable separation of tritium from either of the other two by means of pulse height analysis, although a correction for overlapping of the spectra must be applied. (The spectrum for carbon-14 in Fig. 11, which is very similar to that for sulphur-35, differs from the unquenched spectrum of Fig. 1 because of the higher voltage on the photomultiplier tube necessary for tritium counting.)

The problem was investigated using samples of phenyl-^{3}H-*sec*-butyldisulphide and phenyl-*sec*-butyl-^{35}S$_1$-disulphide which were assayed separately to provide a check and then again after mixing, in the ratio 5 : 1, tritium : sulphur-35, to give phenyl-^{3}H-*sec*-butyl-^{35}S$_1$-disulphide. Sulphur-35 was counted in channel FG under conditions such that tritium made a negligible contribution to background and then tritium plus sulphur-35 were counted in the narrow channel DE. This is essentially the screening method of Okita *et al.* [10] except that in this case the high voltage was maintained constant and the discriminator bias settings varied for each assay. In this way it was found that for channel FG where F = 35 volts, G = amplifier cut off (i.e. gate switched off) sulphur-35 was counted at 70% efficiency and a background of 0.7 c/sec plus <0.1% of the tritium activity. For channel DE where D = 5 volts, E = 10 volts tritium efficiency was 10%, sulphur-35 efficiency was 4% and background was 8.5 c/sec. The precision of the method was assessed by
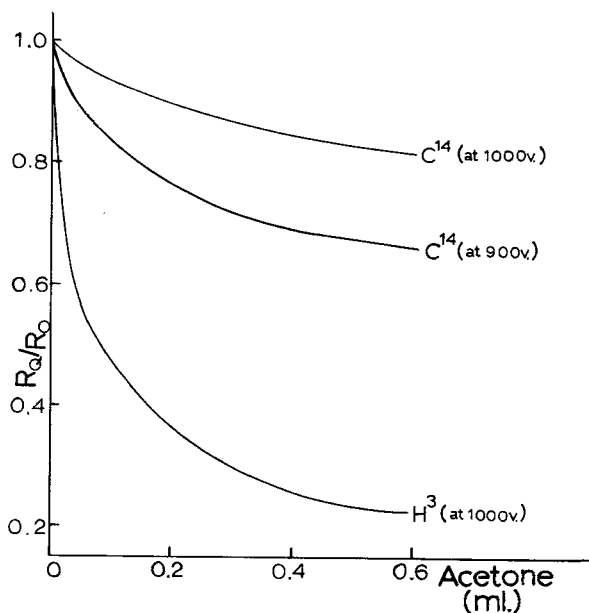

Fig. 12. Relative extents of quenching for carbon-14 and tritium plotted against volume of quench agent.

22 separate assays of the mixed disulphide which gave standard deviation of $\pm 2\%$ for sulphur-35 and $\pm 2.5\%$ for tritium. The mean specific activities were within 4% of those calculated from the singly labelled compounds.

The above experiments relate to unquenched samples. When quenching occurs, however, (Fig. 11) overlapping of the spectra increases eventually to a point where separation is virtually impossible. Even small additions of quench agent give rise to correction problems since the isotopes are quenched to different extents. This effect is clearly illustrated by Figure 12 where the extent of quenching, expressed as the ratio of quenched (R_Q) to unquenched (R_0) count rates, is plotted against volume of quench agent acetone. In the unlikely event of all samples being quenched to exactly the same extent it may be possible to select channel conditions suitable for an accurate assay. Where the extent of quenching is variable but slight, the use of two fixed channels and two internal standards is a reasonable solution [13] which would be applicable to the equipment used for this work.

Use of a channels ratio method of quench correction for doubly labelled samples has been described [11] but scintillation counters with two or preferably three separate channels of pulse height analysis are required. With the equipment used for the present work, re-setting of the discriminator and channel width controls for each sample, three times over, introduced additional errors, which in the authors' opinion were too large and variable to warrant serious consideration of a channel ratio method of quench correction for doubly labelled samples.

ACKNOWLEDGEMENT.

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