J. Lipid Research, July, 1962
Volume 3, Number 3

Scintillation counter for simultaneous assay of H^3 and C^{14} in gas-liquid chromatographic vapors*

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[Received for publication February 13, 1962]

SUMMARY

An instrument for the assay of H^3 and of C^{14} in gas-liquid chromatographic effluents is described. The radioactive analysis is practically simultaneous with the mass-analysis. By the use of coincidence circuitry and pulse-height analysis with discriminators it is possible to measure simultaneously two radioactive isotopes contained in the same sample. Furthermore the addition of a subtracting circuit to the electronic system (described in an Appendix) permits the independent recording of C^{14} and of H^3 counts.

We have previously described a scintillation counter for the continuous monitoring of radioactive vapors emerging from a gas-liquid chromatographic column (1). The need for such an instrument arose naturally from the increased use of gas-liquid chromatography and from the now general application of isotopic tracers in biochemical investigations. Among the several devices suitable for the assay of radioactivity we chose scintillation counting (*a*) because of its known high sensitivity, (*b*) because it is equally suitable for the assay of all radioactive isotopes, and (*c*) because by suitable electronic arrangements it permits the simultaneous measurement of two isotopes.

In our first instrument, the emission of photons in the scintillator was measured with one photomultiplier tube and consequently the level of counts due to natural background radioactivity plus photomultiplier "noise" was relatively high, about 7 second counts per sec (cps). Encouraged by the reliability of our simple instrument, we have developed it further so that the light emission from the scintillator is now measured with two photomultipliers, full use being made at the same time of coincidence circuitry. Pulse-height analysis with discriminator circuits permits the simultaneous measurement of two radioactive isotopes (e.g., of H^3 and C^{14}) contained in the same sample. The arrangement results in great reduction of counts due to random photomultiplier "noise" and background. For a complete understanding of the operation of the new instrument, the present paper should be read in conjunction with our original publication (1) where the principle of the instrument was described in detail.

MATERIALS AND METHODS

Description of the Modified Instrument. All the essential features of our first instrument have been retained including the method of conducting the vapors from the gas-liquid chromatographic (GLC) apparatus through a heated tube to the counter and the method of condensing the vapors in a solution of scintillator, which is made to circulate in a system of tubes and counting chamber by the carrier chromatographic gas. The only important change is the placing of a photomultiplier on each side of the counting chamber (Figs. 1*a* and 1*b*), which is formed by a hole, 4 cm in diameter, in the center of a 1-cm-thick stainless-steel disk of 16.5-cm diameter. The largest part of the channels, in which the scintillator circulates, has now been drilled into this disk, the purpose of the arrangement being the effective cooling of the scintillator by a bulk of metal of high thermal capacity.

The photomultiplier tubes are sealed to the counting chamber, as in our first instrument, through polyethylene sleeves clamped by their flanges with metal rings to the rim of the counting chamber (Fig. 1*b*). Com-

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* Presented at a Symposium on Advances in Tracer Methodology, held in October, 1961, in Washington, D.C. The instrument described is available from the Packard Instrument Company, Inc., LaGrange, Ill.

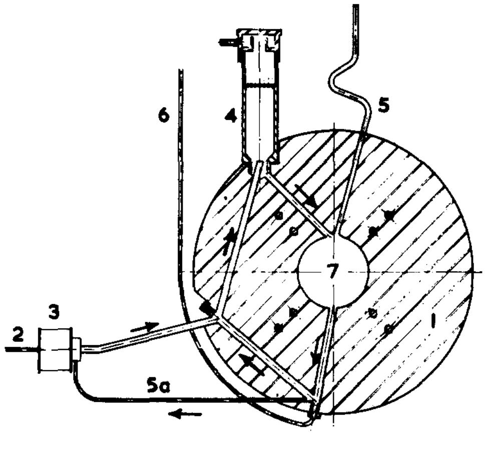
** Present address: The Packard Instrument Co., Ltd., 10–12 St. Johns Road, Wembley, Middlesex, England.

pressible O-rings around the polyethylene sleeves assure a liquid-tight seal around the photomultipliers, the windows of which form the sides of the counting chamber. The depth of this chamber is about 12.5 mm as the photomultipliers are separated from the surface of the metal disk by a thin (about 1.25 mm) ridge of the polyethylene sleeves. Thus the volume of the counting chamber is about 16 ml and the total volume of circulating scintillator 22 ml; it follows that the photomultipliers "see" only about 73% of the circulating scintillator. The heavy lead shielding shown in Figure 1*b* is not needed in areas of normal background radiation.

All parts of the counter, with the exception of the heated tube connecting the counter to the GLC apparatus and the "injection nozzle" (the construction of which was described previously (1)), are placed on a trolley inside a refrigerated box maintained at −5°. By the arrangement of a worm-gear drive, it is possible to move the counter forward and backward for connecting to or disconnecting from the GLC apparatus. The handling of the instrument, filling with scintillator (0.3% diphenyloxazole in toluene), emptying and cleaning, etc., is done exactly as has been described in our previous paper (1).

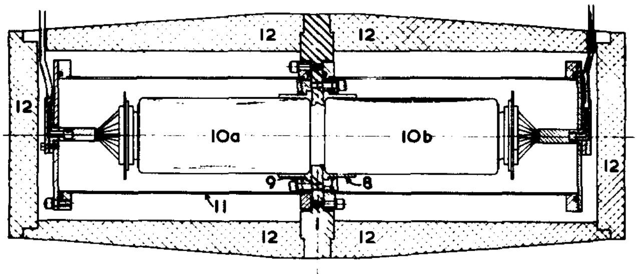
The principle on which this instrument is based has been fully described before and need not be repeated here; but it is worth pointing out that the quantitative condensation of the vapors in the scintillator rests not only on the solubility of the organic vapors in the solvent (toluene) of the scintillator, but also on the fact that the portion of the scintillator that flows through the "by-pass" (Fig. 1*a*, component 5a) to the point of the injection of the vapors is brought to the boiling point of the solvent. The chromatographic vapors are then mixed with a volume of toluene vapor and are condensed together in the cold parts of the channels (Fig. 1*b*, components marked with arrows).

Electronic Circuits Associated with the Instrument. The photomultipliers used have 11 stages.[1] The high voltage to the dynodes of the photomultiplier is applied through a chain of 12 high-stability resistors (each ten megohms) soldered directly to the photomultiplier base. In order to make full use of the potentialities of the instrument two fully transistorized counting channels have been designed each of which can be set independently for the detection of β-rays of different energies. The arrangement, shown in a block diagram on Figure 2, is essentially the same as in the latest model of the Packard Tri-Carb Beta Ray Spectrometer, except that we use fixed-gain amplifiers and we have no upper level discriminator in the high-energy (C^{14}) channel. As this type of electronic circuitry is a familiar one, it is unnecessary to describe it in any detail. By the use of switches it is possible, of course, to carry out single-channel analyses and also to eliminate the anti-coincidence unit in the H^3-channel and thus use both



1a

FIG. 1*a*. End view of main components of scintillation counter: (1) stainless-steel disk 1-cm thick and 16.5 cm diameter with a 4-cm-diameter hole through it forming counting chamber (7); the channels drilled into the disk forming the diamond shaped circuit are 4 mm in diameter; (2) heated tube connecting GLC apparatus with counter; (3) electrical heater surrounding "injection nozzle"; (4) open chimney with light-tight lid for escape of carrier gas; (5) vent-pipe (3-mm diameter) for escape of gas trapped in counter chamber during filling with scintillator; (5*a*) by-pass carrying a portion of circulating scintillator to point of injection of vapors; (6) tube (2-mm diameter) for filling and emptying counter; (7) counting chamber. The arrows indicate the direction of the flow of circulating scintillator.



1b

FIG. 1*b*. Axial cross-section of assembled counter: (8) polyethylene sleeve for sealing photomultipliers to counter disk; (9) clamping device for securing polyethylene sleeve to metal disk and ensuring liquid-tight seal around photomultiplier; (10a and b) photomultiplier tubes; (11) light-tight cover over photomultipliers; (12) lead shielding.

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[1] Type 6097 S; manufactured by E. M. I., Ltd., Hayes, Middlesex, England.

channels for the assay of one isotope only in "integral" counting.

Two ratemeters[2] are used with the equipment, one for each channel, the counting rates being measured as cps. They have 8 counting ranges, the full ranges being 3×10^n and 10×10^n cps, where $n = 0$ to 3. A three-channel moving coil galvanometer[3] is used for taking the records; one channel registers the output of the GLC mass detector (gas-density meter), and the other two that of the H^3- and C^{14}-channel ratemeters.

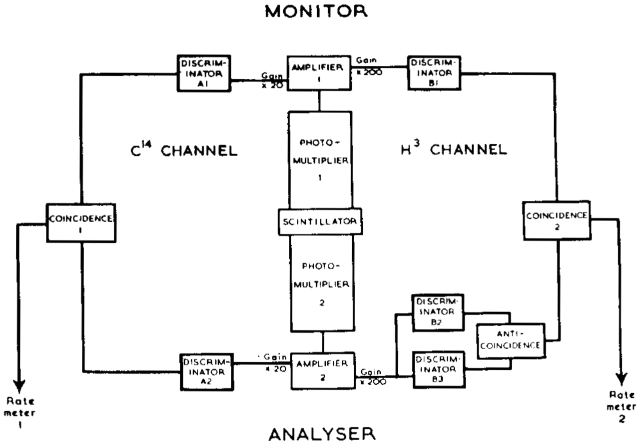

FIG. 2. Block diagram of layout of electronic units of scintillation counter.

RESULTS

The instrument gives highly reproducible results; during one year's operation the maximum variation observed in counting a mixture of C^{14}-labeled fatty acid esters (passed through the GLC apparatus) was ±3%. Much of this variation may be accounted for by errors in pipetting of the samples.

The performance of the instrument may be best illustrated by photographs of original records taken under

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[2] Type N522B; Ekco Electronics Ltd., Southend-on-Sea, England.

[3] Evershed & Vignoles Ltd., Chiswick, London, W.4, England.

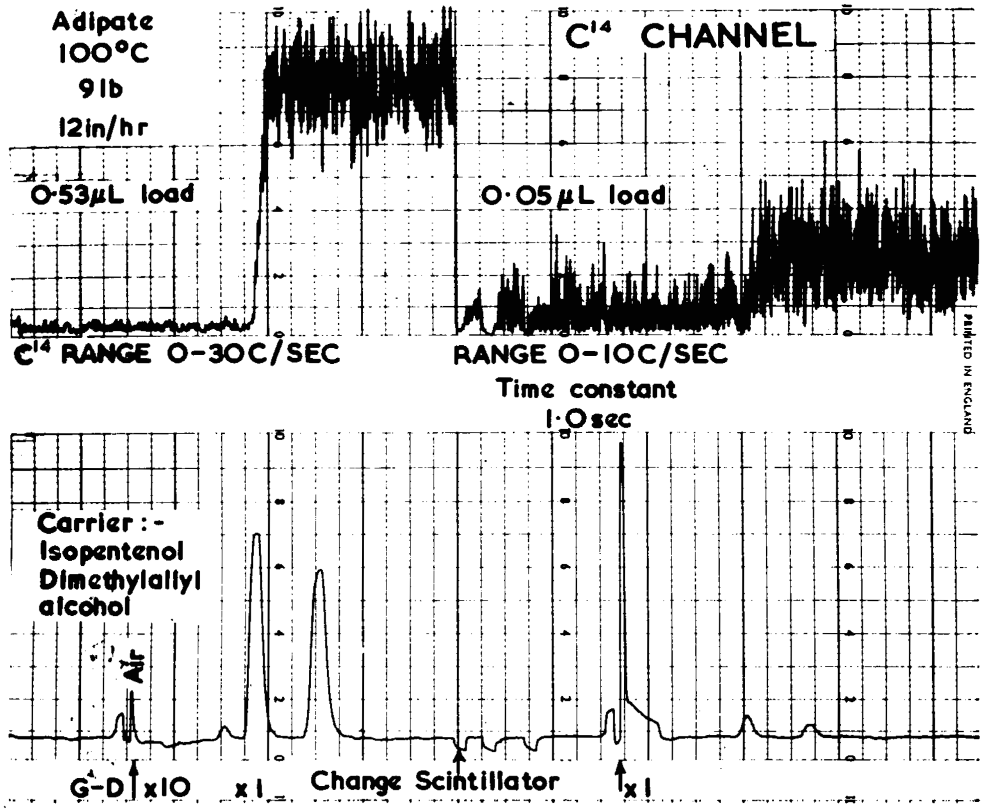

FIG. 3. Repeated analysis of C^{14}-labeled 3-methylbut-2-enol (isopentenol) chromatographed together with "carrier" isopentenol and 3,3-dimethylallyl alcohol. After completion of the first run the counter was washed twice with toluene and then fresh scintillator introduced. G-D ×10 and X1: recording gas-density meter response with one-tenth and full sensitivity respectively.

various conditions. Since outputs of ratemeters are being recorded, and since radioactive decay is a random event, the records show oscillations around a mean value. Although these oscillations could be smoothed out by the use of long integrating time-constants on the ratemeters, such course is impractical because then the radioactivity of a chromatographic fraction leaving the GLC column within a short time interval (e.g., 30–60 sec) would not be recorded correctly within this period. The three-channel recording galvanometer[3] we use is a highly damped instrument; in conjunction with this, the following integrating time constants were found to give the most satisfactory records: 5 sec in the counting range of 0–3 cps, 1 sec in the range of 0–10 and 0–30 cps, and 0.2 sec at all higher counting ranges. The mean counting rate is determined visually by drawing a straight line over the records with the help of a transparent ruler. Since the chromatographic fractions accumulate in the scintillator, the records of radioactivity are of the integral type and therefore the height of each step, showing an increment in radioactivity, represents the total radioactive counts contained in the fraction.

The data of Table 1 show the settings for "integral" counting and for assaying H^3 and C^{14} simultaneously. The background count of the new instrument is satisfactorily low, about one-tenth of that found with the single photomultiplier instrument, thus the range of its sensitivity has been usefully extended to samples of low radioactivity. Over several hours of counting there is usually no perceptible change in the mean level of background counts and a radioactive fraction containing as little as 0.2–0.5 cps above background can be identified with confidence on the 0–10 cps counting ranges provided that the next radioactive fraction does not follow it in less than 1 minute. Figure 3 illustrates two analyses of a mixture of 3-methylbut-2-enol-C^{14} and of 3,3-dimethylallyl alcohol; in the first run 0.53 µl and in the second 0.05 µl of the mixture of the two alcohols was applied to the GLC column. The record shows clearly (*a*) that the radioactivity (C^{14}) was associated only with the 3-methylbut-2-enol, (*b*) that the background counting level was regained after rinsing the counter with toluene, (*c*) that the counts obtained in the second run were one-tenth of those in the first one, and (*d*) that even when a small amount of radioactivity is applied the radioactive fraction produces a well recognizable stepwise increase in the counting level.

TABLE 1. SETTING AND PERFORMANCE OF SCINTILLATION COUNTER FOR C^{14} AND H^3 ASSAY

	C^{14}	H^3
Photomultiplier voltage	1,200	1,200
Amplifier gain (fixed)	×20	×200
Low-voltage discriminator setting (v)	1.2[1] (0.3)[1]	0.3 (0.3)
High-voltage discriminator setting (v)	∞ (∞)	7.0 (∞)
Efficiency of counting (%)	40–50[2]	15–20[2]

[1] These values relative to the setting on the H^3-channel are equivalent to 12 v and 3.0 v when the difference in amplifier gain is taken into account. The values of discriminator settings shown in brackets are those used for integral counting of one isotope.

[2] These values refer to efficiencies measured with standard specimens passed through the GLC apparatus, and when the instrument was set for the simultaneous assay of C^{14} and H^3. In integral counting when only one isotope is measured, the efficiency of C^{14} counting is nearly 80% and that of H^3 about 28%; these values depend also on the photomultipliers in use.

With the settings of discriminators shown in Table 1, less than 0.1% of the H^3-counts appear in the C^{14}-channel, which—excepting specimens of very high H^3 and low C^{14} content—may be neglected (cf. Fig. 4). On the other hand, about 16% of the C^{14}-counts are also recorded in the H^3-channel (Fig. 5). Since virtually no H^3-counts appear in the C^{14}-channel, the true H^3-counts are obtained by subtracting 16% of the C^{14}-counts from the counts observed in the H^3-channel.

The fraction of the C^{14}-counts that necessarily must appear in the H^3-channel may be subtracted from the H^3-records electrically. This can be done particularly easily when the radioactive assay is carried out with ratemeters, which translate pulses into a flux of current. By the use of a subtracting circuit, described in the Appendix, it is possible to subtract from the current of the H^3-channel ratemeter an adjustable proportion of the output of the C^{14}-channel ratemeter. Figure 6 illustrates that when such a subtracting circuit is included in our system, the mean counting level in the H^3-channel remains unchanged even when C^{14}-labeled fractions of high radioactivity are being assayed. The effect of this electrical subtraction is that the standard error of the C^{14}-counts is superimposed upon the standard error of the counts of the H^3-channel. If, therefore, the counting rate in the H^3-channel is low, but that in the C^{14}-channel is high, the standard error of the counting rate in the H^3-channel will reflect the standard error of the C^{14}-channel rather than that of the H^3-counts (see also Appendix). For this reason, this practice is advocated only when the counting range of the C^{14}-ratemeter is not higher than three times that of the H^3-ratemeter, although the counting range of the H^3-ratemeter could be 30–100 times higher than that of the C^{14}-ratemeter.

Figure 7 shows a simultaneous C^{14} and H^3 analysis when 16% of the C^{14}-counts were automatically sub-

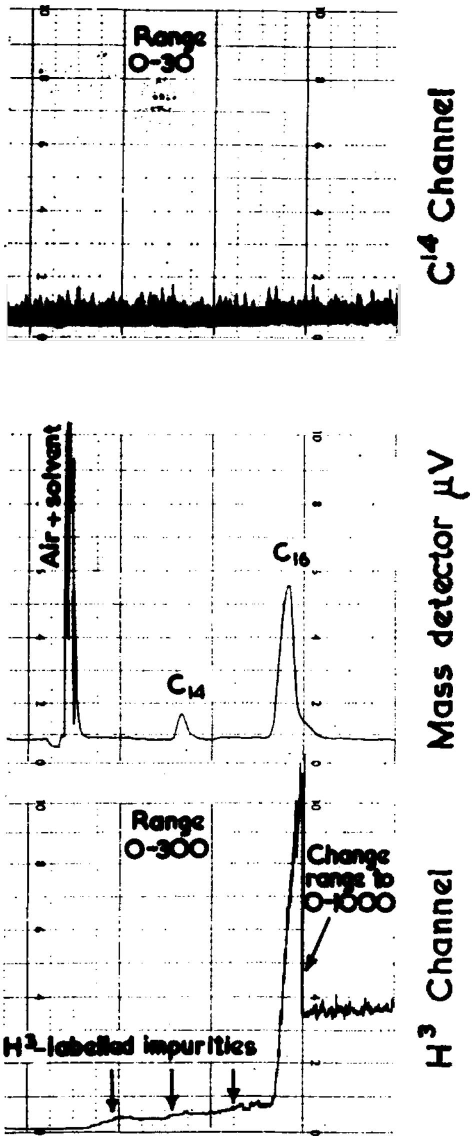

FIG. 4. Analysis of methyl H^3-palmitate. Settings of C^{14}-gate and of H^3-window were as shown in Table 1. The total recorded H^3 counts were 360 cps; there was a just perceptible rise (<0.3 cps) of counts in the C^{14}-channel the sensitivity of which was set initially ten times higher than that of the H^3-channel.

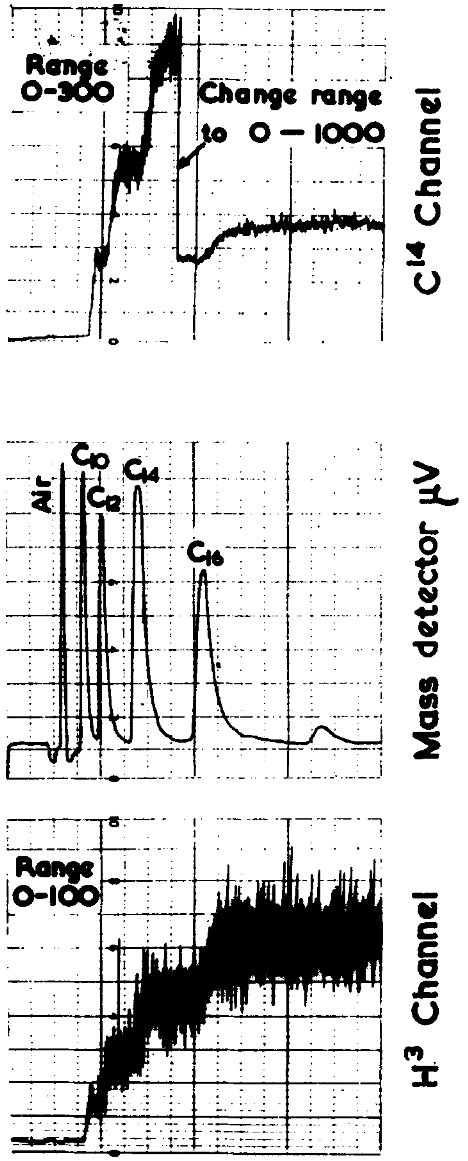

FIG. 5. Analysis of a mixture C^{14}-labeled methyl esters of C_{10}, C_{12}, C_{14}, and C_{16} fatty acids. C^{14}-gate and H^3-window as in Table 1. Sixteen per cent of the C^{14} counts were also registered in the H^3-channel the sensitivity of which was set three times higher than that of the C^{14}-channel.

tracted from the H^3-channel. The radioactive fractions appearing on the H^3-record before palmitate are all due to H^3-labeled impurities (cf. Fig. 4).

As the successive radioactive fractions accumulate in the scintillator during the analysis of a complex mixture, one might, of course, be forced to use counting ranges of lesser and lesser sensitivity. This can, however, be avoided by the changing of the scintillator during a chromatographic run, as it requires only about 20 seconds to remove the scintillator and to fill the counter again. When the scintillator is changed without rinsing the counter with clean toluene, the counting rate usually drops to about 10% of the previous rate. Figure 8 shows such an instance; after the third radioactive fraction, the scintillator was changed and the radioactivity of the fourth fraction was measured with the same sensitivity as the activities of the previous three fractions. The scintillator may be changed repeatedly during the analysis of a sample containing several radioactive fractions.

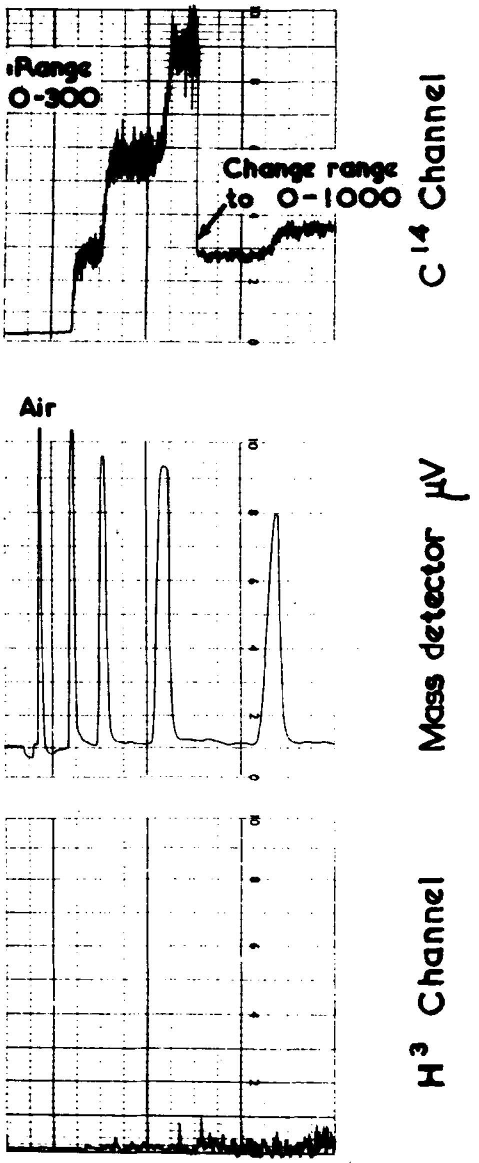

FIG. 6. Analysis of the same mixture of C^{14}-labeled methyl esters (and same amount) as shown in Fig. 5 with subtracting circuit in operation (see also text). The analyses shown in Fig. 5 and 6 were carried out with two different columns in the GLC apparatus; note reproducibility of counts.

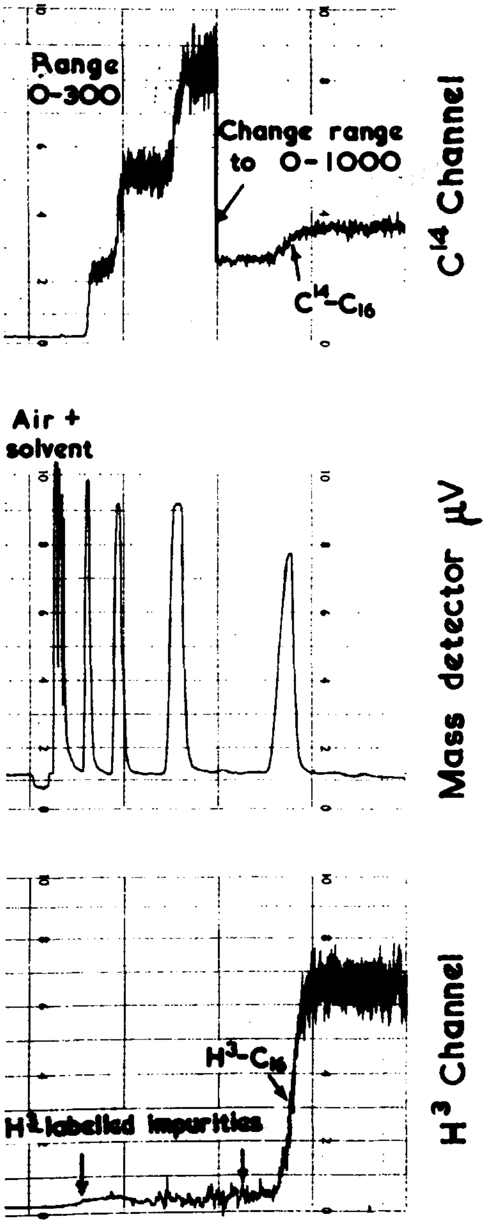

FIG. 7. Simultaneous C^{14} and H^3 analysis with subtracting circuit in operation (see also text). The counts recorded in the H^3-channel are due to H^3 alone.

DISCUSSION

An instrument of this type (at first fitted with one photomultiplier only) has been in use in our laboratory for four years now and has proved its reliability. The improvement described here allows the measurement either of H^3 or of C^{14}, or of both simultaneously, with equal ease and with high efficiency in all compounds that may be separated by gas-liquid chromatography. Quenching of the counts, attributable either to the slow stripping of the stationary phase (polyester type, or Apiezon vacuum grease) from the GLC columns, or to a nonradioactive chromatographic fraction following a radioactive one, has never been observed. So far we have used the equipment for measuring the radioactivity of esters of mono- and dicarboxylic acids, of alcohols and their derivatives, and of hydrocarbons. The method is particularly suitable for the analysis of radioactive substances of high volatility (e.g., of low-boiling alcohols), which would be impossible to study by paper chromatography without their previous conversion to nonvolatile derivatives. The general application of the technique is not only in biosynthetic

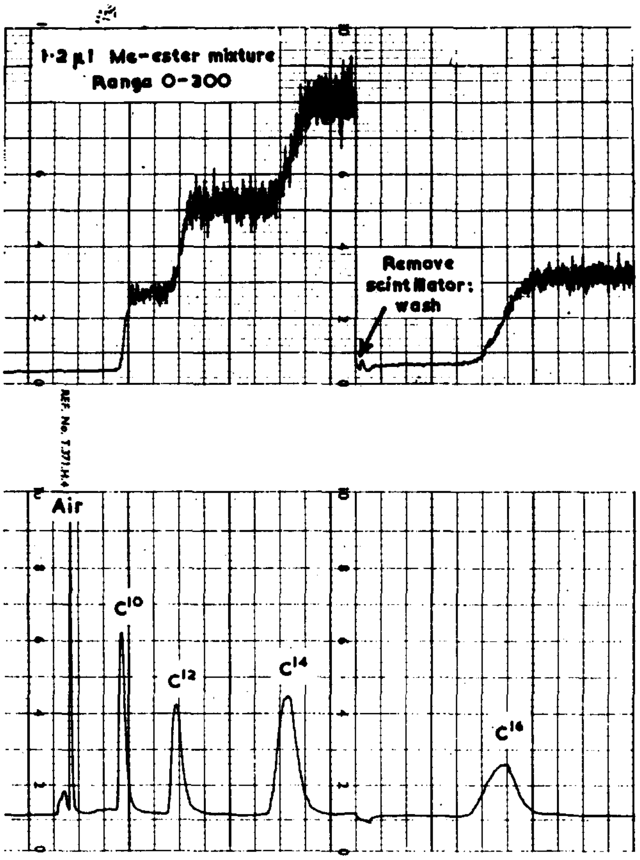

FIG. 8. Analysis of C^{14}-labeled methyl esters. Record illustrates that the scintillator can be renewed during a chromatographic run in order to maintain required sensitivity of counting range. After the third radioactive fraction, the scintillator was withdrawn, the counter was rinsed once with toluene, and then fresh scintillator was introduced.

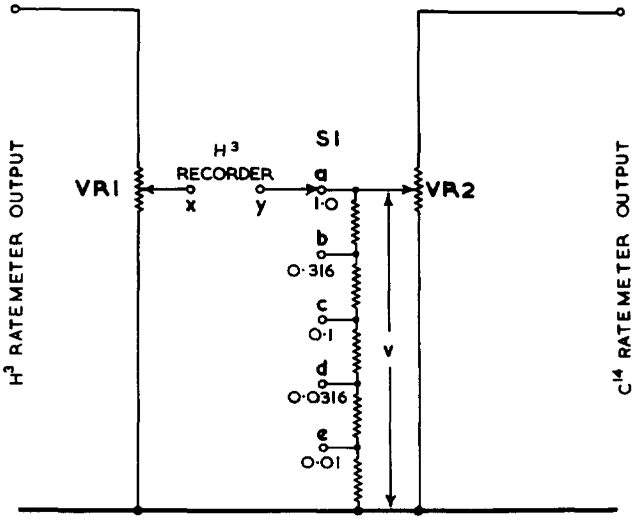

FIG. 9. Subtracting circuit for the elimination of C^{14}-counts from the record of H^3-channel. Figures marked at positions *a* to *e* on switch S1 show the fraction of the output of the C^{14}-ratemeter (as modified by variable resistor VR2) appearing at *y*.

and turnover studies, but also in the analysis of synthetic labeled substrates used in biochemical investigations. Experience has taught us not to trust implicitly the radioactive purity of any compound of whatever origin. Figure 4 shows only a portion of the analysis of an allegedly pure H^3-palmitate; in fact the specimen contained no less than 15 radioactive impurities accounting for about 25% of the radioactivity; several other examples of this kind could be quoted.

REFERENCE

1. Popják, G., A. E. Lowe, D. Moore, L. Brown, and F. A. Smith. *J. Lipid Research* 1: 29, 1959.

APPENDIX

ELECTRICAL SUBTRACTION OF C^{14} PULSES FROM H^3-CHANNEL COUNTS DURING SIMULTANEOUS ASSAY OF THE TWO ISOTOPES WITH THE AID OF RATEMETERS (by A. E. Lowe)

In the apparatus described in the main body of this paper, the energy spectra of C^{14} and H^3 are separated by a pulse-height discriminator, or "gate," for the C^{14}-channel, and a pulse-height analyzer, or "window," for the H^3-channel. The output from each channel is measured with a separate ratemeter. The gate of the C^{14}-channel can be set so as to exclude all H^3-counts from it, but a fraction of the C^{14}-disintegrations are necessarily counted in the H^3-window. In order to register on the H^3-recorder counts proportional only to the H^3-content of the specimen, a portion, $\beta \times C$, of the output (C) of the C^{14}-channel ratemeter must be subtracted from the output of the H^3-channel ratemeter, β being the fraction of the C^{14}-counts in the H^3-window. However, as the selected counting ranges of the two ratemeters may be different, the subtraction from the

TABLE 2. SETTINGS OF SWITCH S1 OF SUBTRACTING CIRCUIT DEPENDENT ON COUNTING RANGES OF C^{14}- AND H^3-RATEMETERS

Selection of Positions on Switch S1	Ratio of C^{14}- and H^3-Ratemeter Counting Ranges
	C^{14}: H^3
a	10:1
b	3:1 or 10:3
c	1:1
d	1:3 or 3:10
e	1:10

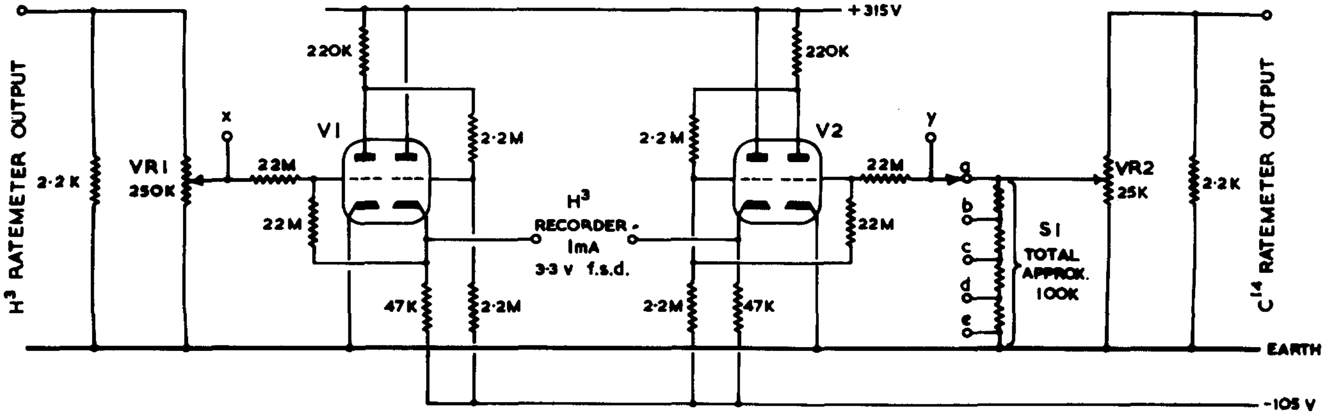

FIG. 10. Combination of subtracting circuit with two cathode-followers for driving a moving-coil recording galvanometer. VR1, VR2, S1 and points *x*, *y* correspond to components shown in the circuit on Fig. 9. Valves V1 and V2 are double triodes, type ECC8 or 12AT7.

output of the H^3-ratemeter must be equal to $\beta \times C \times R_c/R_H$, where R_c and R_H are the selected counting-rate ranges (3×10^n or 10×10^n) of the C^{14}- and H^3-ratemeters respectively. The circuit of Figure 9 will do this subtraction. The variable resistor VR1 in the circuit is used to adjust the calibration of the H^3-recorder and VR2 to subtract a current proportional to the C^{14}-component of the H^3-channel counts. The position of switch S1 must be selected according to the ratio of R_c to R_H as set out in Table 2. Of course, in a double tracer experiment, when the C^{14}-component of the H^3-channel has been subtracted, the H^3-recorder will register only a fraction of the ratemeter output. Consequently, without some further arrangement, the full range of the recorder could not be used; this is undesirable. However, if the ratemeter is modified so that its current output is linearly proportional to a pulse rate of up to twice the full counting rate of any of the selected counting ranges, it becomes possible to subtract from the output of the H^3-ratemeter a current as high as that produced by a normal full-scale count and the whole range of the recorder can be used. The same end could be achieved by doubling the sensitivity of the recorder so that it registered full-scale deflection for half the ratemeter output. As our ratemeters could be modified easily, we have adopted the former course.

The circuit shown in Figure 9 will operate correctly only with recorders that need a negligible driving power; e.g., with potentiometric recorders. When moving-coil galvanometers, which need driving power, are used, it is necessary to insert, between points *x* and *y* of the circuit, cathode-followers. A composite circuit of this type, suitable with commonly used moving-coil recording galvanometers, is shown in Figure 10.

The subtracting circuit will over- or undercompensate slightly when $R_c/R_H = 3 \times 10^{n_1}/10 \times 10^{n_2}$ or $10 \times 10^{n_1}/3 \times 10^{n_2}$, because both of these ratios are being treated in the circuit as $\sqrt{10} \times 10^{(n_1-n_2)}$. The maximum error introduced this way, with settings for the C^{14}-gate and H^3-window shown in Table 1, is not more than ±2.56%. The arrangement of the subtracting circuit necessitates, at present, the manual selection of the appropriate position of switch S1 whenever the ratio of R_c to R_H is altered during a chromatographic run. It would be possible to replace S1 with an automatic switching system and also to have an automatic change of R_c and R_H.

For the practical setting up of the subtracting unit, the counter is filled with scintillator containing 0.1–1 μc of H^3, and the C^{14}-gate is set so that only normal background count is registered on the C^{14}-channel ratemeter (cf. Table 1). The variable resistor VR1 is then adjusted until the H^3-recorder registers the correct counting rate, which—bearing in mind the modification to the ratemeter or to the recorder—should be twice the rate shown on the ratemeter. After changing to a C^{14}-source (0.1 μc) the same counting range is selected on the H^3-channel ratemeter as is needed on the C^{14}-channel and switch S1 is set to position *c*. A "H^3-window" is selected that does not permit more than about 16% of the C^{14}-counts being registered in the H^3-channel. The variable resistor VR2 is then adjusted until the H^3-channel recorder registers only the normal background count.

We wish to thank our colleagues, Mr. L. Brown, Mr. A. Hewett, and Mr. F. A. Smith, for their assistance in designing and constructing the instrument. The illustrations were prepared by Miss Monica Sinton. Figure 3 is an illustration from the work of Miss Hermione Hellig of this Unit.