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Scintillation counter for the measurement of radioactivity of vapors in conjunction with gas-liquid chromatography

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SUMMARY

The construction and performance of a liquid scintillation counter for the measurement of radioactive vapors is described. The instrument is used in conjunction with a gas-liquid chromatographic instrument for the measurement of C^{14} contained in chromatographic vapors. With a single photomultiplier tube 50 per cent of the C^{14}-disintegrations are counted and recorded parallel with the chromatographic analysis. The records obtained are of the integral type. The resolving power of the counter is such that radioactive fractions needing only 25 seconds to pass through the chromatographic analyzer (gas-density balance) may be clearly distinguished from other radioactive fractions following in 1 minute or less.

The instrument described in this paper was designed to measure the radioactivity of C^{14}-fatty acids emerging as vapors from a gas-liquid chromatographic column and to record the radioactive disintegrations parallel with the analytical chromatographic record. The need for such an instrument arose out of work in this laboratory on the biosynthesis of fatty acids and other volatile substances from C^{14}-labeled precursors in the course of which a resolution of mixtures of the substances and assay of the C^{14}-content of the individual components became necessary. To accomplish such a task by available methods proved time-consuming and in some instances misleading or inaccurate. Paper chromatography of fatty acids has limited powers of resolution; liquid-liquid partition chromatography (e.g., on a reversed phase column) offers a wider scope, but a complete analysis of a complex mixture together with assay of isotope in each chromatographic fraction may take a week or longer. The gas-liquid chromatographic technique of James and Martin (1) is undoubtedly the method of the greatest power of resolution for volatile substances. But even in this method, if a mixture of C^{14}-labeled fatty acids (or their methyl esters) are resolved, the collection of the fractions followed by plating of the samples for radioactive assay presents problems, not the least among which is the loss by volatilization of the methyl esters of acids with a short or medium-length carbon chain from planchettes. Collection of fractions by bubbling the effluent vapors and carrier gas from a gas-liquid chromatographic column directly into a solution of scintillator, followed by counting in a suitable scintillation counter, has been used by us with success; the method is simple and sensitive but lacks resolution. It may be used only when the retention volume of each radioactive chromatographic fraction is known precisely.

Specimens may be encountered in which a radioactive component of high specific activity may be present in such small quantities that even the gas-density balance, one of the detectors used in gas-liquid chromatography (2), may not show them distinctly or such components may overlap with some well-recognized fatty acid. In such instance the radioactivity may erroneously be attributed to the wrong substance. For these reasons it was thought desirable to develop an instrument that will record continuously the radioactivity of vapors in conjunction with gas-liquid chromatography. It will be seen from the records presented that the power of our instrument to differentiate between radioactive fractions emerging from the gas-liquid chromatographic instrument is as great as the

power of the columns employed; the radioactivity of a fatty acid ester that takes only about 20 to 30 seconds to pass through the analytical detector (gas-density balance) may be correctly assigned to that ester and is clearly separated from another ester emerging 1 minute or less afterwards. The principle of the instrument and a simple prototype has already been described briefly (3).

PRINCIPLE OF THE INSTRUMENT

The principle of the instrument is very simple and depends on the injection of the chromatographic vapors together with the carrier gas (N_2) into a continuously circulating and cooled solution of phosphor (diphenyloxazole in toluene or xylene). The organic vapors, by virtue of their solubility in the solvent and their high boiling point, become condensed and dissolved in the toluene or xylene; the carrier gas, on the other hand, escapes to the atmosphere. If the substance condensed in the solution of phosphor is radioactive, then the light emission (scintillation) excited by the radiation can be measured with a photomultiplier tube.

Let us imagine a circuit consisting of tubes *A*, *B*, *C*, and *D* as shown in Figure 1; at the junction of *D* and *A* there is a capillary inlet (*I*) and at the junction of *A* and *B* a chimney (*E*) open to the atmosphere. If this circuit were filled with liquid, this would leak out through the capillary *I*, but if gas is blown through the capillary, the gas pressure prevents this: the gas bubbles rise in tube *A* and escape through *E*. Since the gas bubbles displace liquid in tube *A*, the liquid will circulate in the direction of the arrows (Fig. 1). When the circuit is made of tubing with an internal diameter of 4 mm. and gas is blown through *I* at the rate of 40 to 160 ml. per minute (gas flow rates commonly used in gas-liquid chromatography) the circulation of liquid is very vigorous, as can be shown by the addition of dyes to the liquid; complete mixing takes place in our final instrument within 5 seconds when a gas flow of 40 ml. per minute is used. If the liquid in the circuit is a solution of a phosphor (liquid scintillator) and radioactive vapors are injected at *I*, the radioactive material will pass around the circuit and excite the emission of light photons, which can be measured with a photomultiplier placed at the junction of tubes *B* and *C* (indicated by the dotted circle in Fig. 1). This is the prototype of our instrument, which was constructed and found to function correctly (3). The output from the photomultiplier, after amplification, is measured with a ratemeter and continuously recorded. Since the radioactive material accumulates in the solution of phosphor, the record obtained is of the integral type, a distinct advantage over the usual type of chromatographic record.

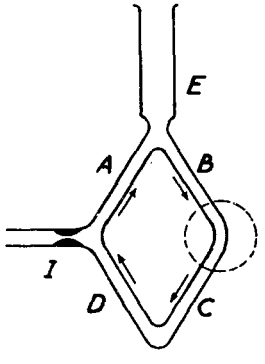
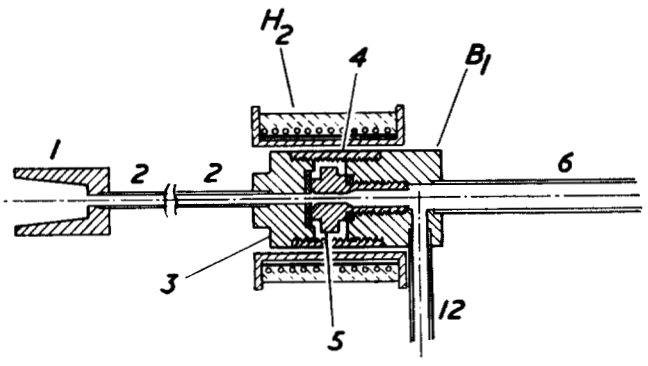


FIG. 1. Schematic representation of principle of scintillation counter for measuring radioactivity of vapors. For description see text.

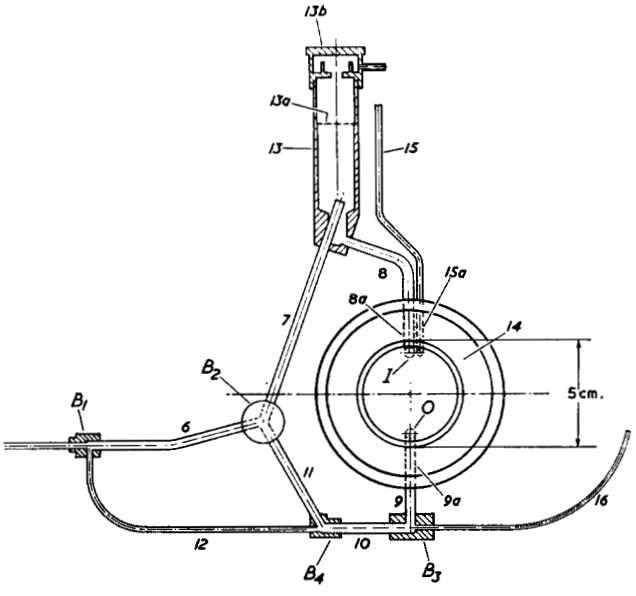
DESCRIPTION OF THE INSTRUMENT

In the construction of the instrument several conditions had to be met: (*a*) the solution of the scintillator and photomultiplier had to be kept cold, while the chromatographic vapors—in order to avoid their condensation before they reach the scintillator—had to remain at the temperature (up to 200°C) of the chromatographic instrument up to the point of their injection into the scintillator; (*b*) in order to obtain maximum efficiency there must be no light-absorbing surfaces interposed between the scintillator and the window of the photomultiplier and the whole window area of the photomultiplier must be used "to see" the scintillations; the photomultiplier must see the largest possible fraction of the circulating scintillator; (*c*) gas bubbles must not appear in front of the photomultiplier, as these would prevent some of the light photons—by internal reflections—from reaching the sensitive photocathode; and (*d*) the instrument must be light-tight. The prerequisite to all these conditions, an efficient removal of organic vapors from the carrier chromatographic gas and rapid mixing in the scintillator, was assured from our preliminary experiments with a prototype instrument.

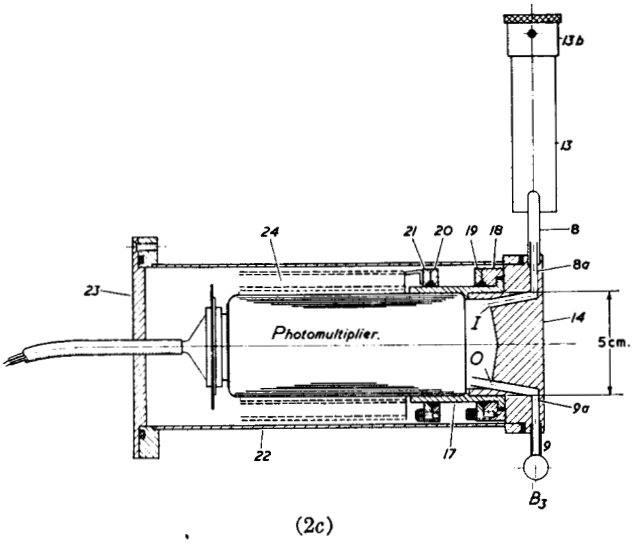
Condition (*a*). In order to prevent condensation of the organic vapors before they are injected into the scintillator, the vapors and carrier gas are conducted from the detector (gas-density balance) of the gas-liquid chromatographic instrument to the counter through an electrically heated tube, which is kept 10°-20°C above the temperature of the gas-density balance (2). The whole instrument, which has a large heat capacity, is kept inside a refrigerated box (−5°C);



(2a)



(2b)



(2c)

FIG. 2. Constructional details of scintillation counter for measuring radioactivity of vapors. Components shown on more than one illustration are given the same numbering: (*a*) heated tube connecting counter with gas-liquid chromatographic instrument and injection nozzle; (*b*) cross-section of counter through counting chamber; (*c*) side elevation of counter. For description of individual components see text. Figures 2*b* and 2*c* were drawn to scale.

only the heated connecting tube and the point of injection of the vapors is outside the cold box.

Condition (*b*). In order that the photomultiplier may see the largest possible fraction of the circulating scintillator, the portion of the circuit between tubes *B* and *C* shown in Figure 1 was expanded into a shallow circular chamber of very nearly the same diameter as the diameter of the photomultiplier tube. The volume of this chamber is about 18 ml.; the total volume of scintillator required to fill the instrument is 25 ml. and therefore the photomultiplier sees 72 per cent of the circulating liquid. To avoid light-absorbing surfaces between scintillator and photomultiplier, the window of the photomultiplier acts as the front wall of the circular chamber.

Condition (*c*). To avoid loss of light photons reaching the photomultiplier by internal reflection of light within bubbles, the gas and vapors are injected into the scintillator outside the "counting chamber"; but even so, when a high rate of gas flow is used in the chromatography, the circulation of liquid in the counting instrument is so vigorous that the stream of fluid may drag gas bubbles from the escape funnel (Fig. 1, *E*) into the counting chamber and eventually a gas-lock may develop at the top of the counting chamber. This has been prevented by placing tube *A* (which corresponds to tube 7 in Fig. 2*b*) at a slope, rather than vertically, and by extending it into the chimney (*E*) to a height about 2.0 cm. above the level of the orifice of tube *B*. Further, a vent pipe has been inserted into the top of the counting chamber, through which gas dragged into this chamber may escape, or may be sucked out with a syringe (see "Operation of the Instrument" below).

Condition (*d*). With the exception of the photomultiplier and the polyethylene sleeve used for its attachment (see below), the functional parts of the instrument have been made of stainless steel and the photomultiplier tube is sealed in a light-tight and moisture-proof metal casing.

CONSTRUCTION OF THE INSTRUMENT

The construction of the instrument is illustrated in Figures 2*a*, 2*b*, and 2*c*, and its assembly in Figure 3. The counter is connected to the detector of the gas-liquid chromatographic instrument through a stainless steel tube (1.5 mm. bore; 15 cm. long; Fig. 2*a*; com-

ponent 2), which at one end is hard soldered into a brass socket of a standard taper joint (Fig. 2*a*; component 1), and at the other end into the hole of a screw provided with a hexagonal head (Fig. 2*a*; component 3). The socket fits the cone, which is the outlet of the chromatographic detector, and the screw fits into the sleeve (Fig. 2*a*; component 4), which surrounds the injection nozzle. Before the connecting tube is soldered into the brass socket, a woven-glass insulating sleeve is pulled over the tube. As this insulating sleeve can be stretched or compressed, the soldering can be done without damage to it. An insulated copper-constantan thermocouple is pushed under the insulating sleeve over which a heating element H_1 (not shown on Fig. 2*a*) is wound out of nichrome wire (30 standard wire gauge) insulated with woven-glass sleeving. The total resistance of this heater is 4 ohms.

The connecting tube leads to the injection nozzle fitted to block B_1 (cf. Figs. 2*a* and 2*b*; component 4), the construction of which is shown in detail on Figure 2*a*. The actual nozzle is a 6BA screw with a hexagonal head and drilled out as illustrated (Fig. 2*a*; component 5); it is screwed into block B_1. Copper gaskets are used in front and at the back of the injection nozzle to ensure gas-tightness.

The chromatographic vapors enter the scintillator, circulating in tubes 6 to 12 and the counting chamber, through the 1 mm. bore hole of the nozzle. This particular construction of the injection nozzle (and of block B_1) was arrived at after testing four other simpler designs, all of which proved unsatisfactory mainly because the scintillator tended to crystallize out around the capillary inlet, causing blockage which was difficult to clear or because the capillary inlet could not be heated adequately to prevent early condensation of vapors. The present arrangement permits quick dismantling and cleaning of the injection nozzle and connecting tube, although this has not been necessary during more than 6 months of daily operation. Also we had no sign of tailing of radioactive peaks that could be attributed to premature condensation of vapors, whereas such troubles were met in earlier designs.

Before the connecting tube is screwed into the sleeve (Fig. 2*a*; component 4) surrounding the injection nozzle, an electric heater (H_2) wound on a metal reel is fitted over the sleeve and heats block B_1 and the parts of the connecting tube that screw into B_1. This heater, like that for the connecting tube, is made, over woven-glass insulation, from nichrome wire (30 standard wire gauge; 6 ohms). A thermocouple is inserted under the insulation of this heater also. The heaters are supplied with current from suitable tappings on two low-voltage transformers. These transformers are fed from a duplex variable transformer.

The liquid scintillator circulates in tubes 6 to 12 (Fig. 2*b*); the circuit includes the counting chamber 14. These tubes are joined together by hard-soldering into four stainless steel blocks B_1 to B_4, into the gas escape chimney 13 and into the block of the counting chamber 14, all drilled at the appropriate angles. The liquid scintillator leaving tube 10 divides into two streams, into tubes 11 and 12. Tube 12, a bypass of the main stream of scintillator, carries liquid just to the tip of the injection nozzle and has an internal diameter of 2 mm., whereas all the other tubes (6 to 11) are of 4 mm. internal diameter. This bypass has been introduced in order that the injection of hot vapors may be made away from the cooled parts of the instrument and in order that all the circulating phosphor may not be heated; in Figure 2*b* the parts to the right of block B_1 are inside and those to the left are outside the refrigerated box.

The gas-escape chimney (Fig. 2*b*; component 13), 7.5 cm. long, internal diameter 1.6 cm., is machined out of a solid rod and has a narrower well (8 mm. diameter, 1.5 cm. deep) at its bottom. The holes, into which tubes 7 and 8 are soldered, open into this well; tube 7 protrudes into, and slightly beyond, the well to a total length of 2 cm. Thus the orifice of tube 8, in which liquid moves downward, is below the level of the scintillator fluid where the gas bubbles escape from tube 7. The arrangement prevents gas bubbles from being dragged into the counting chamber. Inside the chimney, 2 cm. from the top, a fine platinum wire mesh soldered to a ring (component 13*a*) has been inserted in order to break up the splashing of the scintillator. The chimney is covered with a screw-cap lid (component 13*b*) on the inside of which there is a simple light trap. A side tube screwed into the cap allows the carrier chromatographic gas to escape and is usually connected through a polyethylene tube to a gas-flow meter.

The block of the counting chamber 14, to which the photomultiplier tube is attached, is machined on a lathe from a stainless steel disk 8.5 cm. in diameter and 3.6 cm. thick. The internal diameter of the counting chamber is 4.4 cm., its outer diameter 5 cm., and its depth at the side is 8 mm. The bottom of the chamber has been machined to the shape of a shallow cone and highly polished to provide a reflecting surface for light. The inlet and outlet for the scintillator fluid is provided by the channels (4 mm. diameter) (Figs. 2*b* and 2*c*; components 8*a* and 9*a*) drilled to form an elbow at the upper and lower edges of the chamber. A polyethylene tube has been inserted tightly into the

exit hole *O*, and protrudes into the counting chamber by 8 mm. The insertion of this tube ensures that the liquid entering through the inlet hole (*I*) will not stream down the bottom of the counting chamber, but will mix with its contents. At the upper edge of the counting chamber and parallel with the inlet channel (component 8*a*) there is a similar size channel (component 15*a*) which opens into the vent pipe (Fig. 2*b*; component 15). A stiff polyethylene tube with a slight upward curvature is inserted into the orifice of this ventilating channel and reaches to the front upper corner of the counting chamber and allows the air to escape from the counting chamber during filling of the instrument with scintillator (see also "Operation of the Instrument" below). The counter is filled and emptied through tube 16 (Fig. 2*b*), internal diameter 2mm., which is soldered into block B_3.

The polyethylene sleeve (Fig. 2*c*; component 17), 3 mm. thick, which secures the photomultiplier to the counting chamber has a 2 mm. thick internal ridge which sits on the edge of the wall of the counting chamber. This ridge acts as a cushion between the glass photomultiplier and the metal edge of the counting chamber and prevents damage to the tube during assembly. Thus the depth of the counting chamber at its side walls is 10 mm. The polyethylene sleeve is secured by its flange to the chamber block with a ring (Fig. 2*c*; component 18) screwed to the block. Although polyethylene does not dissolve in either toluene or xylene (solvents for the scintillator), it swells slightly on prolonged exposure to these solvents. In order to assure a tight fit of the sleeve around the counting-chamber wall and the photomultiplier, O-rings have been fitted around both and squeezed tightly between the V-grooves formed by two rings appropriately shaped (Fig. 2*c*; components 18, 19, 20, and 21). No leakage of scintillator has been observed with this sealing arrangement over several months of operation. It is seen that the window of the photomultiplier tube forms the front wall of the counting chamber; thus the only loss of light is by absorption in the scintillator itself.

A light-tight cover (Fig. 2*c*; component 22), made of a brass cylinder and painted black, fits over the photomultiplier. It is secured to the block of the counting chamber by a screw thread and is sealed off with a rubber gasket. This cover is fitted with a lid (Fig. 2*c*; component 23) which has a hole in its center for the cable to the photomultiplier. A rubber sleeve over the cable ensures a light- and air-tight fit.

A double-walled cylinder made of perforated tin sheet and lined with filter paper (Fig. 2*c*; component 24) is filled with silica-gel drying agent and placed around the photomultiplier before the light-tight cover is screwed down.

The whole counter assembly is surrounded by a lead shield, 2.5 cm. thick (Fig. 3). The lead shield was

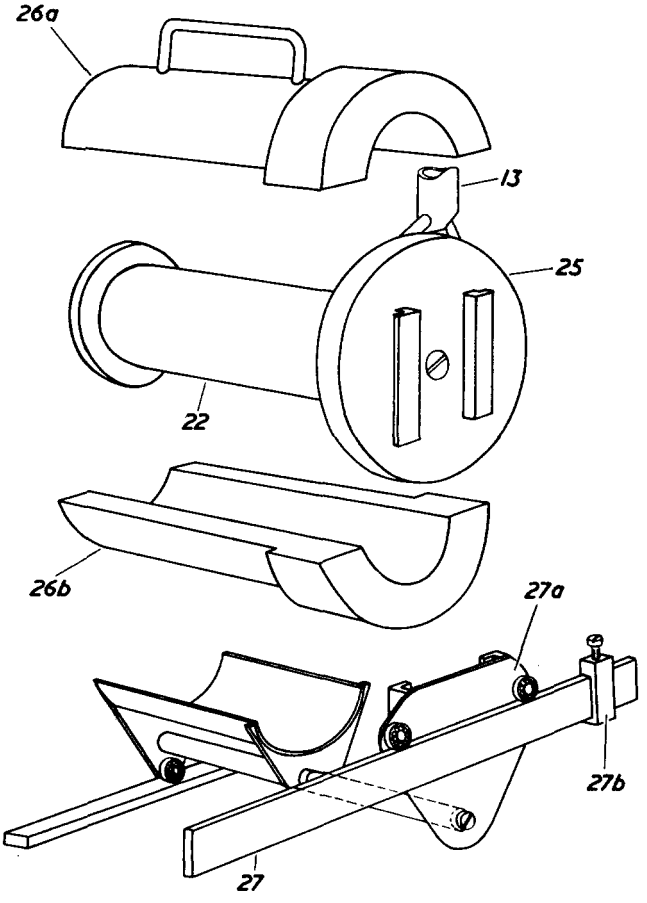


FIG. 3. Components of lead-shield and cradle for scintillation counter.

made in three parts: (*a*) a disk to which the counting chamber is secured with a stout screw; this lead disk (Fig. 3; component 25) is provided with a handle (not shown in Fig. 3) and two vertical slides which secure the counter to a cradle (see below) inside a refrigerated box; and (*b*) a cylinder slit into two halves along its length (Fig. 3; components 26*a* and *b*). As the photomultiplier lies horizontally, the lower half of the lead cylinder acts as a cradle for the instrument and is mounted inside a refrigerated box on sliding rails (Fig. 3; component 27). It is the sliding-rail arrangement of this cradle which carries a vertical plate (component 27*a*) to which the counter assembly is secured by the vertical slides attached to the lead disk described above.

When the counter is fitted into the refrigerated box, the cradle is rolled to the back of the box and the

counter, secured to the lead disk, is lowered into the cradle; the top half of the lead shield is then put into position. The whole assembly is then rolled forward until the injection nozzle protrudes out of the refrigerated box through a hole cut into the front of the box; the counter is now secured in this position by tightening the clamping screw of the sliding arrangement (Fig. 3; component 27*b*) inside the box. The cylindrical electric heater (H_2) is now pushed over the injection nozzle and the tube connecting the counter to the gas-density balance is screwed tightly into the sleeve surrounding the injection nozzle.

The arrangement of the sliding cradle has been made in order that the counter may be placed quickly and easily into a predetermined position inside the refrigerated box and without causing damage to the protruding pipes joined in block B_1. The aim in the construction of the lead shielding and cradle—as of other parts too—has been an ability to dismantle the instrument quickly if a need for maintenance should arise.

The refrigerated box, which houses the counter, measures $43 \times 43 \times 43$ cm. on the outside; the thickness of its insulated walls is 7 cm. The cooling coil on the inside of the box is connected to a single-stage compressor unit through flexible metal tubing. The thermostat to the refrigeration unit is set to $-5°C$ and responds to the air temperature in the box. With this setting, the temperature of the counter and of the scintillator liquid is maintained steadily at 0°C while the hot vapors and carrier gas are injected into it.

The box rests in an angle iron frame on two rollers and can be moved forward and backward on these with a worm gear. The box rests at such height that the socket of the connecting tube is opposite the outlet cone of the gas-density balance; by the use of the worm gear it can be quickly and easily attached to it, or disconnected from it.

The layout of the whole assembly is shown in Figure 4. The output from the photomultiplier tube, after amplification, is measured with a ratemeter.[1] This ratemeter was modified so as to provide for purposes of recording two ranges in the ratio of 10×10^n:3×10^n cps., where n may be selected with a switch to be 0, 1, 2, 3, or 4. Both the $10\times$ and $3\times$ ranges are simultaneously recorded in our instrument and provide two levels of sensitivity for the measurement of radioactivity. The range of the instrument has been further extended by the addition of an automatic switch: when the output from the ratemeter reaches four-tenths of the level of any of the 10×10^n ranges, and therefore the output is beyond the recording limits of the 3×10^n range, the switch automatically changes this to the $3 \times 10^{(n+1)}$ range. A further automatic switch provides protection for the 10×10^n range by disconnecting it from the ratemeter when its reading exceeds 30 per cent overload. As a consequence of this arrangement of recording it is hardly possible to meet a situation when a correct record of the counting rate would not be made. These operational details will be further illustrated by actual records shown in Figures 6 and 7. The records are taken with a three-channel recorder: two of the channels are used for the radioactive record and the third for the analytical chromatographic record, which registers the response of the chromatographic detection device, which in our instrument is a modified gas-density balance which will be described in a future communication.

[1] Type 1172, EKCO Electronics, Ltd., Southend-on-Sea, England.

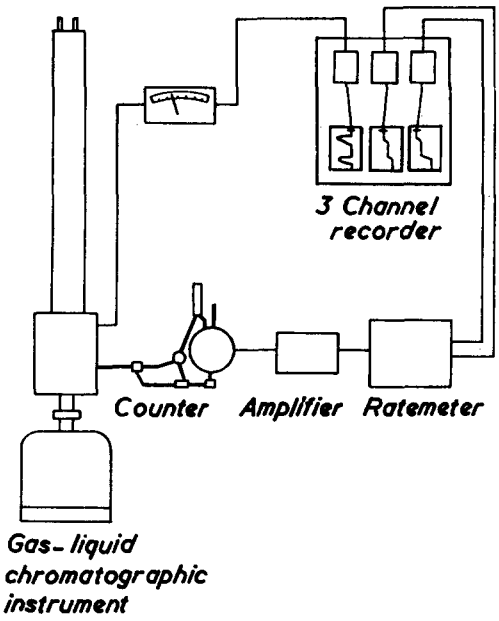


FIG. 4. General layout of scintillation counter, gas-liquid chromatographic apparatus, and recording instruments.

OPERATION OF THE INSTRUMENT

For setting up the counter for measurements the refrigerated box containing the counter is moved forward on its rollers until the socket of the connecting tube fits snugly over the cone of the outlet of the chromatographic instrument through which the chromatographic gas is already flowing at the required rate. A simple manifold, fastened to one of the sides

of the refrigerated box and consisting of three 3-way taps in a line, a syringe, and three reservoirs, provides an easy means for filling the counter with scintillator solution, for removing the used scintillator, and for washing out the instrument. The arrangement is shown in Figure 5. Reservoir R_1, holding the solution of scintillator, is kept inside the refrigerated box; R_2 and R_3 are outside this on a shelf under the box. For filling the counter, taps T_2 and T_3 are turned so as to open the reservoir of scintillator solution (R_1) to syringe S_1; 25 ml. of scintillator solution are now drawn into the syringe and tap T_3 is turned off by rotating it by 45°. Tap T_2 is now turned around by 180° and tap T_1 is turned so as to open the manifold from the syringe to the counter-filling tube. The contents of syringe S_1 are now emptied into the counter. It will be realized that at the moment when the level of the scintillator in the counter reaches above the level of the gas-vapor injection nozzle, the gas flow will force the scintillator liquid up in tubes 6 and 7 into the gas-escape chimney from where the liquid will flow through tube 8 into the counter chamber (cf. Fig. 2*b*). Without the vent pipe 15 it would not be possible to fill the counter chamber completely with scintillator because the flow of liquid from the gas-escape chimney down through tube 8 would prevent the escape of air from the counter chamber. When all the scintillator has been expelled from syringe S_1, any air that might have been trapped at the top of the counting chamber is drawn out with syringe S_2, which is attached to the vent pipe of the counter chamber with a polyethylene tube. The hollow piston of syringe S_2 has two holes, one at the bottom and one at the top; when the air is being drawn out of the counting chamber, the hole at the top of the piston is closed with the tip of a finger. The sign of complete withdrawal of all air from the counter chamber is when a solid column of liquid, uninterrupted by any gas bubbles, appears below syringe S_2. Now by lifting the tip of the finger from the hole of the piston, the liquid drawn up into S_2 will return to the counter. After this manipulation the counter can be operated for several hours without a gas lock developing in the counter chamber. Before the chromatographic run can be started, the electric current for the two heaters, one around the connecting tube and the other around the injection nozzle, are turned on and the variable transformers controlling these currents are so adjusted that the thermocouple reading of the temperature of the connecting tube registers about 20°C above the temperature of the chromatographic instrument and that of the heater for the injection nozzle at about the boiling point of the scintillator solvent (toluene; 110°C). With the heaters we use, the appropriate heater currents are obtained at 6 to 8 V for the connecting tube heater and with 12 to 14 V for the injection-nozzle heater when the chromatography is done at 200°C.

Since during the filling of the counter the scintillator is exposed briefly to light, there is some phosphorescence induced which requires a few minutes to decay. A 5- or 10-minute record is therefore taken before the start of a chromatographic run in order to obtain the stable background level of counting.

With the particular type of photomultiplier employed,[2] it was found that an E.H.T. setting of 1600 V

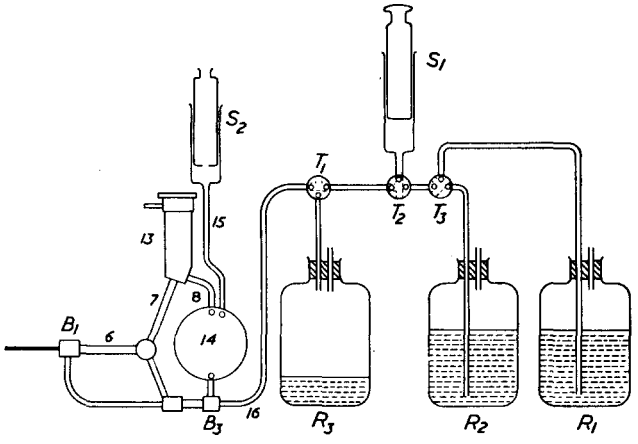


FIG. 5. Manifold used for filling and emptying scintillation counter. For details see text.

to the photomultiplier, together with a discriminator bias voltage of 25 to 30 V on the ratemeter, provided the optimum conditions for counting: the background plus photomultiplier noise being 7 to 10 cps. and the counting efficiency about 50 per cent. It should be pointed out that in the calculation of efficiency no allowance was made for the fact that the photomultiplier can "see" only 72 per cent of the scintillator.

It follows from the background counting level that the lowest useful and most sensitive range that may be selected on the ratemeter for recording purposes is the 100:30 ($10 \times 10:3 \times 10$) setting. In order to obtain a record of radioactivity with the minimum of delay after the entry of a radioactive substance into the counter, the integrating time constant of the ratemeter must be kept short: 1 second in the counting ranges of 100:30 cps. and 0.2 second at all higher ranges should not be exceeded.

[2] VMP 13/44 (Twentieth Century Electronics, Ltd., New Addington, Surrey, England).

On account of these short time constants, the records obtained show the oscillations characteristic of the randomness of radioactive disintegrations. However, these oscillations are around a mean value that can be read without any difficulty (cf. Fig. 6). Only after the background count has settled to a steady level is the sample to be analyzed put on the chromatographic column. During the loading of the column the stream of gas through the counter must be maintained, otherwise the scintillator would flow out through the connecting tube. As our instrument is used in conjunction with a gas-density balance, during the loading of the sample column the gas stream through the reference column is kept flowing and maintains the circulation of the scintillator in the counter.

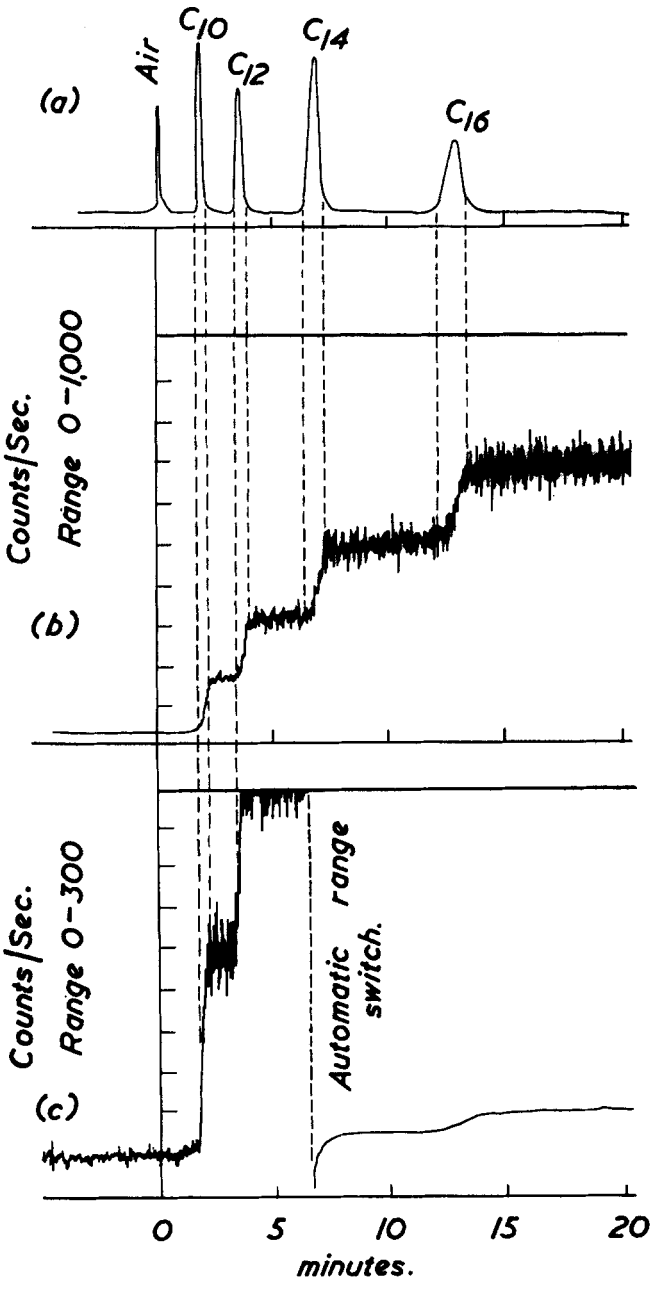


FIG. 6. Gas-liquid radiochromatogram obtained from the analysis of the methyl esters of four C^{14}-labeled fatty acids on ethylene glycol-polyadipate ester column at 197°C. (*a*) Analytical record; (*b*) and (*c*) simultaneous records of radioactivity made at two levels of sensitivity. Total load 1.25 mg. of esters containing 1362 disintegrations/sec. Analytical record taken with one-tenth the normal sensitivity of the gas density balance. See also text.

PERFORMANCE OF THE COUNTER AND SOME OF ITS APPLICATIONS

We considered that the instrument would fulfill its functions satisfactorily if (*a*) the radioactive vapors elicit the response of the scintillation counter within a few seconds after the beginning of the response of the chromatographic analyzer; if (*b*) the width of the record of the radioactive band (integral shaped record) is identical with the width of the analytical (differential) chromatographic record; if (*c*) radioactive peaks following one another very quickly are clearly distinguished; and if (*d*) the response of the counter at all levels of radioactivity, within the ranges of the ratemeter (up to 30,000 pulses per second), is proportional to the radioactivity introduced.

Some of these demands of performance may be best illustrated by records taken during the analysis of a standard mixture of the methyl esters of four C^{14}-labeled fatty acids (C_{10}, C_{12}, C_{14}, and C_{16}) on two columns and in different quantities. The first record (Figs. 6*a*, *b*, *c*) was taken during chromatography of the four acids on a column with a polyester stationary phase (ethyleneglycol adipate) at 197°C and with a total gas flow of 100 ml. per minute (50 ml. per minute through sample column). Under these conditions of very fast analysis, the first fraction (methyl decanoate) required only about 25 seconds to pass through the gas-density balance. It can be seen that the record of radioactivity for each methyl ester is a well-defined step and the time taken for the development of each step corresponds exactly to the time required for the passing of each methyl ester through the density balance. No delay is discernible on the records between the beginning of the analytical record and the beginning of the corresponding radioactive record. By observation of the progress of recording and by timing with a stop watch, however, we found that in every instance the beginning of the radioactive record is delayed after the beginning of the record from the gas-density balance by exactly 10 seconds. This delay is no doubt

due to the time required for the vapors to leave the density balance and for the mixing of the vapors in the scintillator. The analysis shown in Figure 6 is considered to be a severe test of the performance of the instrument on account of the high C^{14}-content of the specimen; the load on the column was so large (1.25 mg. of the esters) that the output from the gas-density balance had to be recorded with one-tenth the usual sensitivity and the total radioactivity contained in the four acids was 1362 disintegrations per second. From this and many other analyses it appears that the resolving power of the scintillation counter is as good as the resolving power of the columns used in chromatography. Figure 6*c* also illustrates the operation of the automatic switch described earlier: on the 3×10^2 range the limit of the range was reached after the appearance of methyldodecanoate and the automatic switch changed this range to 3×10^3 in the middle of the recording of the radioactivity of methyl tetradecanoate.

Another analysis carried out on a slow column with Apiezon-L as stationary phase is shown in Figures 7*a*, *b*, and *c*. As in Figure 6, record *a* represents the analytical chromatographic record from the response of the gas-density balance, and graphs *b* and *c* show the parallel measurements of the radioactivity of the methyl esters at two levels of sensitivity. In this analysis the same mixture of C^{14}-labeled methyl esters was used as in the analysis shown in Figure 6, except that only 430 μg. of the methyl esters were applied to the column. The four methyl esters, C_{10}, C_{12}, C_{14}, and C_{16} were contained in a mixture in the proportions of 1:1:2:2 (w/w), but the specific activities of the C_{14} and C_{16} esters were only about one-half of those of C_{10} and C_{12}. The total radioactivity contained in the 430 μg. of the applied specimen was 466 disintegrations per second, which, when counted on a planchette at negligible thickness with an end-window Geiger-Müller counter (G.E.C. Research Laboratories, Type EHM2, window thickness 1.2 mg. per cm.^{2}), gave 1680 cpm. The total number of counts found in the four methyl esters by the scintillation counting of the vapors was 235 cps., giving a counting efficiency of 50 per cent. From the slower chromatographic analysis on the Apiezon-L column the correct "integral" shape of the tracings of radioactive peaks may be more readily appreciated than from the record shown in Figure 6. It can be seen that the half-value of each step on the radioactive record corresponds very closely to the peaks on the analytical record and thus the radioactivity may be assigned to the corresponding methyl ester identified by the analytical record. As the radioactivity of each ester is given by the height of each step in the record, the specific activity of individual fractions may be calculated if the sensitivity of the analytical detection device is known. As this can be determined by appropriate calibrations, the specific activities of the various fractions may be deduced. For example, the area under the curve representing methyl tetradecanoate corresponds to the response of our gas-density balance to 144 μg. of the ester which gave 66 cps. in the scintillation counter. Thus the specific activity of the methyl tetradecanoate was 458 cps. per mg. ester (or 916 disintegrations per second per mg. ester).

A comparison of the records shown in Figures 6 and 7 indicates also that the response of the counter is strictly proportional to the amount of radioactivity applied to the chromatographic column. In the analysis shown in Figure 6, 1.25 mg. of the esters were used; these gave a total count of 680 cps. in the four fractions. In the second analysis (Fig. 7) 430 μg. of the same esters gave 235 cps., in excellent agreement with the expected figure.

The instrument can also be used as the only chromatographic detection device for specimens containing radioactive substances in so low concentrations that they could not be detected with a gas-density balance nor even with the far more sensitive ionization-chamber detector of Lovelock *et al.* (4). We have encountered such an instance in recognizing, entirely by their radioactivity, a new series of unsaturated branched-chain acids and other volatile substances synthesized enzymically from C^{14}-labeled mevalonic

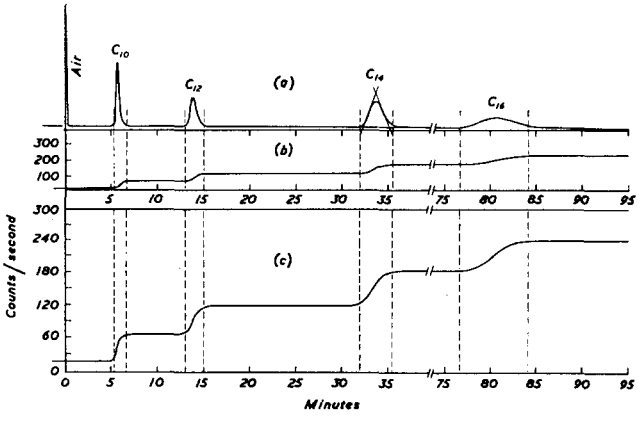


FIG. 7. Gas-liquid radiochromatographic analysis of the methyl esters of C^{14}-labeled fatty acids on Apiezon-L column at 197°C. Total load 430μg. of esters containing 466 disintegrations/sec. (*a*) Analytical record; (*b*) and (*c*) records of radioactivity taken at two levels of sensitivity.

acid (to be published). In Figure 8 is reproduced a composite tracing of a record obtained from the analysis of 5 μg. of methyl esters prepared from a lipid extract of *Chlorella vulgaris* grown from a small inoculum for 3 weeks under photosynthetic conditions in the presence of $C^{14}O_2$ of high specific activity.[3] By the use of the ten times more sensitive range of the counting device, an entirely satisfactory analysis could be carried out with a total of 0.5 μg. of the esters (applied to the chromatographic column in toluene). By the use of markers, introduced together with the C^{14}-labeled esters, most of the radioactive components could be assigned—according to their retention volumes—to well-recognized fatty acid esters, although these had not elicited any response from the gas-density balance.

It should be also pointed out that the slight pressure (approximately 10 cm. of toluene) under which the gas-density meter operates when the counter is attached to it, and the fluctuations of this pressure, owing to the bubbling of gas through the scintillator liquid, have no noticeable effect on the performance of the density meter provided it is correctly balanced.

The day-to-day behavior of the scintillation counter is very constant and analyses of specimens may be reproduced accurately even several months apart.

There is a limitation of the sensitivity of the instrument in that, with the narrow charts (3 in.) used in the recording, radioactive components may be recognized only if the total radioactivity in the component is not less than 2 per cent of the range selected on the ratemeter. Thus, if one selects the 100× range for recording, then 2 counts/second above background—or above the previous level of counting—is readily discernible, but on the 1000× range, 20 cps. are needed to show up as a distinct change in the level of counting. Of course the simultaneous recording at the 10×10^n and 3×10^n ranges increases the sensitivity, so that in the two examples given above, 0.6 and 6 cps., respectively, above previous counting levels may be recognized. After some experience has been gained with the instrument, a change in the counting rate of not more than 1 per cent of the full-scale range may be appreciated. The above limitations are, however, only technical in nature as by the use of larger recorders with a full-scale deflection of 10 inches the sensitivity of the instrument could be improved. Likewise, by employing two photomultiplier tubes on opposite sides of the counting chamber, and connected to a coincidence circuit, a gain in counting sensitivity with lower background count could be achieved although the size and cost of the counter would increase proportionately.

No quenching of the scintillations has been observed by the vapors leaving the chromatographic columns, nor by the small amounts of the stationary phase (Apiezon-L or ethyleneglycol adipate polyester) stripped off the columns. Even after the counting of very active samples (in the region of 10,000 cps.), the background counting rate returns to normal after rinsing out the instrument three times with 25 ml. of clean toluene. This rinsing is usually done through the filling manifold (Fig. 5): the used scintillator is withdrawn into syringe S_1 and ejected into the refuse bottle (R_3), then toluene is drawn up from the reservoir R_2 and injected into the counter as described for filling with the scintillator. Usually 30 to 60 seconds are allowed for the circulation of each washing in the counter for thorough cleaning.

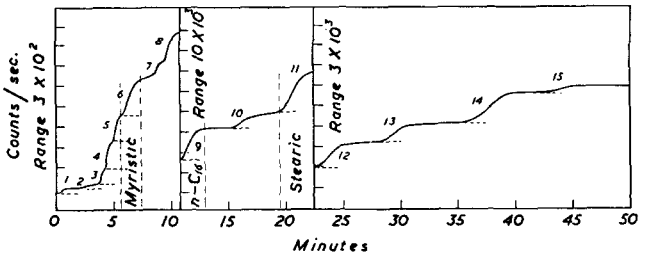


FIG. 8. Gas-liquid radiochromatogram of the methyl esters of fatty acids obtained from *Chlorella vulgaris* grown in the presence of $C^{14}O_2$ of high specific activity. Stationary phase ethylene glycol-adipate polyester; temp.: 197°C. Total load 5 μg. Chromatographic fractions 1, 3, 4, 5, 7, 8, and 10 not definitely identifiable; they are probably various branched-chain acids and/or acids with odd numbers of carbon atoms, e.g., fraction 10 is probably a saturated C_{17} acid. The following fractions could be identified: 2, dodecanoate; 6, tetradecanoate (myristate); 9, palmitate; 11, stearate; 12, oleate; 13, linoleate; 14, eicosanoate; 15, eicosa-mono-enoate. No further component appeared beyond 15.

During an analysis, when several radioactive components have already accumulated in the counter, it is sometimes desirable to change the scintillator in order to return to the more sensitive ranges of the counter; this can be done during a suitable interval between chromatographic fractions as the operation requires only about 1 minute. Washing with clean toluene is usually omitted in such an instance; the height of the next radioactive peak is simply taken from the counting level obtained after the change of the scintillator. This counting level is usually 5 to 10 per cent of that observed before the changing of the scintillator.

A large number of biosynthetic specimens have already been studied by our instrument and it has been found that the most useful ranges for recording were

[3] We are indebted to Dr. J. Catch of the Radiochemical Centre, Amersham, Bucks., for the gift of the lipid extract.

provided by the 10×10^2 and 3×10^2 scales as in the record shown in Figure 8. So far we had to use the most sensitive (100:30) scales very rarely. Of course it is an advantage in arriving at the appropriate selection of ranges for the recording if one knew in advance the approximate number of counts put on the chromatographic columns; this is most conveniently determined by counting an aliquot of the specimen.

Although we have used the instrument so far only for counting C^{14}, there is no reason why it should not be equally suitable for counting tritium or any other radioactive isotope contained in an organic vapor. The instrument has been calibrated with a standard sample of tritio-hexadecane and the efficiency of counting was 20 per cent when electronic noise was 3 cps. and the background counting rate 12 cps.

We are indebted to Messrs. E. K. Cole, Ltd., Southend-on-Sea, Essex, England, for loan of equipment during the development of the counter.

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