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REVIEW

RADIO-GAS CHROMATOGRAPHY

S BABA

Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo (Japan)

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CONTENTS

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ECD	Electron-capture detection
FID	Flame ionization detection
GC	Gas chromatography
HPLC	High-performance liquid chromatography
2-ME	2-Methoxyethanol
MS	Mass spectrometry
RAD	Radioactivity detection
RGC	Radio-gas chromatography
RHPLC	Radio-high-performance liquid chromatography
RT	Residence time
SARD	Synchronized accumulating radioisotope detection
S/N	Signal-to-noise ratio
TCD	Thermal conductivity detection
TCSF	Total counts of successive fractions
TLC	Thin-layer chromatography

1 INTRODUCTION

Radio-gas chromatography (RGC) has been an important analytical tool for many years, the main developments being actively carried out between the early 1960s and mid-1970s However, few papers have been published during the last decade The history and performance of RGC and a discussion of the choice of methods in RGC were well summarized by Roberts [1] Matucha and Smolková [2] surveyed comprehensively original papers published on RGC up to 1976. However, after 1976, only one review [3] has been published In the annual fundamental reviews published in Analytical Chemistry, RGC was dealt with in the subtitle of 'Radiochemical Detectors', which has been missing since 1982 The main reason for this may be attributable to the tendency since the mid-1970s for high-performance liquid chromatography (HPLC) to be used instead of gas chromatography (GC)

In this paper, recent developments in RGC are described, and a method based on a new detector assembly, named synchronized accumulating radioisotope detector (SARD), is presented with emphasis on the analysis of weak β-emitters in organic and biochemical applications

2 RADIO-GAS CHROMATOGRAPHY

2 1 Description of the method

RGC consists of three processes, separation of radioactive components by a GC column, mass detection and radioactivity detection Although RGC was

initially carried out on packed columns, systems equipped with capillary columns have been developed recently.

Mass detection is usually performed by thermal conductivity detection (TCD) or flame ionization detection (FID), electron-capture detection (ECD) and mass spectrometry (MS) can also be exploited because of their higher sensitivities. The instruments for mass detection and radioactivity detection (RAD) are usually arranged in parallel. The effluents from a GC column are split in a suitable ratio, the minor portion passes through the mass detector, and the major is transferred for RAD. However, with tritiated compounds with a high specific radioactivity, the splitting ratio should be reversed

Methods for the detection of radioactivity in the effluents from a GC column fall into two categories, namely 'continuous methods', in which the radioactivity is monitored continuously, and 'discontinuous methods', in which the components in the effluents are trapped as they emerge from the column for separate radioassay Continuous methods have the advantage that the RAD trace is produced simultaneously with the mass detector trace The major criticism of continuous methods is that they are inherently less sensitive than discontinuous methods because of the relatively short counting time. Although the discontinuous methods are laborious and time-consuming, they have higher sensitivity Moreover, trapping techniques can be simple and inexpensive, and a liquid scintillation counter can be used for the radioactivity determination In most situations, however, continuous methods for RGC are used and hence trapping techniques are less frequently utilized.

In the early stages of development of RGC, all techniques for radioactivity detection, including Geiger–Muller counters, gas flow proportional counters, ionization chambers and scintillation counters, were used Most RGC systems reported in the current literature use gas flow proportional counters for RAD, although a few systems use an anthracene flow cell

Based on the chemical form in which the effluents from a GC column are radioassayed, RGC can be classified into two types, those with and those without decomposition of the effluent The techniques in which the effluents are directly transferred for RAD have not been generally accepted, however, because the radioactive components often condense on the inside wall of the transfer line and detector, resulting in high background counts In addition, RAD is particularly sensitive to high temperature The decomposition step is now almost invariably carried out by a combustion technique although hydrocracking [4] was examined in the early days

Fig 1 illustrates the typical arrangement of an RGC system which is most commonly used today

The oxidation–reduction (or combustion) tube is a quartz tube packed with copper oxide and iron fillings, placed in an electric furnace The inner volumes of most combustion tubes used in RGC are several millilitres The temperature of the inside of the furnace is between 700 and 800 °C The extent of deterio-

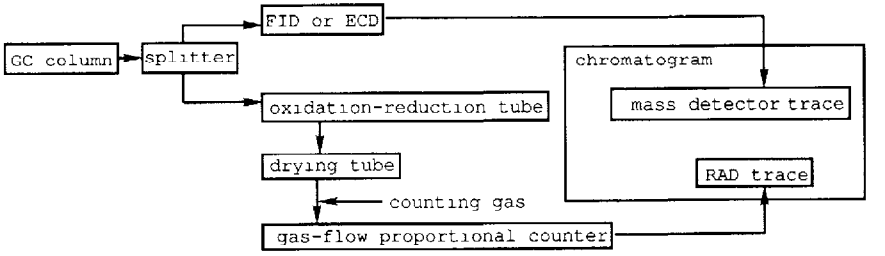


Fig 1 Schematic diagram of RGC system of the most common type

ration of the copper oxide can be followed by its colour change (from black to red) A tube packed with 10 g of copper oxide retains a sufficient capability for combustion until the volume of the injection solvent amounts to 200 μl [5] Labelled compounds in the effluents are oxidized to carbon dioxide and water If ^3H is present, the tritiated water produced is then reduced to $^3H_2$ The effluent containing $^{14}CO_2$ and/or $^3H_2$ is mixed with counting gas (methane, propane or carbon dioxide) and then passed through a gas flow proportional counter

The counter tube is a metal tube with an inner volume usually between 10 and 30 ml The optimum voltage applied to the counter tube can be found by use of external radiation sources

The flow-rates of the carrier and counting gases should be strictly regulated, because the detection sensitivity is severely influenced by the composition and flow-rate of gas passing through RAD

The radioactivity in RGC can be recorded by two methods analogue recording by means of a rate meter or digital recording of the radioactivity per certain time period (sampling time) as resulting in a histogram The latter is more convenient for the quantitation of radioactivity and is more accurate However, almost all the RGC systems use analogue recording, with a few exceptions [6,7], because digital recording suffers from severe statistical fluctuations of counts owing to a limited counting time, especially with samples of low radioactivity The time constant of the rate meter is set usually between several and ten seconds

The volume of the counter tube, the composition of gas passing through the counter tube and its total gas flow-rate vary widely Table 1 summarizes RGC systems of the gas flow proportional counter type

2 2 *Performance*

In designing or operating a gas flow proportional counter for RGC, one of the most important problems to consider is the relationship between the chromatographic resolution and detection sensitivity This problem will be discussed briefly using the case of a gas flow proportional counter as an example

TABLE 1

SURVEY OF RADIO-GAS CHROMATOGRAPHIC SYSTEMS (COMBUSTION AND GAS FLOW PROPORTIONAL COUNTER TYPE)

Counter tube volume (ml)	Gas flow-rates in the counter tube (ml/min)	Counting efficiency (%)		Background counts (cpm)	Ref
		^{3}H	^{14}C		
10	H_2 30, CH_4 20	30	88	35	4
10	H_2 22 5, CH_4 22 0	98 4	102 4		8
10	N_2 23 8, CH_4 17 6		~80		9
5×30[a]	He 60, CH_4 240	56	92	75	10
12	Ar 95, CO_2 5	64	94 5	1 0	11
20	He 54, propane 6				12
10[b]	He 80, H_2 10, propane 10				13
18[b]	Ar 27, H_2 3, CO_2 2				14
~5[b]	He 59, O_2 1, CO_2 6		70	3-5	6
7×10[a b]	He 50, CH_4 variable (100-550)	~65	95	38	7

[a]See Section 4 1
[b]For capillary RGC

The volume of a mass detector is negligibly small compared with the peak volume occupied by a radioactive component emerging from a GC column On the other hand, for radioactivity measurement a counter tube having a much larger volume must be used to obtain a high sensitivity The intensity of a radioactive peak recorded on a radiochromatogram is a function of residence time (RT) multiplied by the amount of radioactivity present in the counter tube Here, RT is defined as the time required for a specified point in the sample to pass through the counter tube and can be calculated by dividing the volume of the counter tube by the total flow-rate of gas passing through the tube

Fig 2 shows the radiochromatograms of [^{14}C]hexadecane obtained under various RT (for RT in this instance, see Section 4 1) [7]

Although the RT should be as small as possible to maintain the chromatographic resolution, any reduction in RT is accompanied by a corresponding reduction in the sensitivity, as illustrated in Fig 2 Therefore, when monitoring radioactivity, a compromise between the chromatographic resolution and detection sensitivity must be made, its exact nature depending on the analytical requirements Snyder and Kirkland [15] discussed the similar states of matters for radio-HPLC (RHPLC) Where the maximum resolution of GC is required, for example, in the analysis of a complex mixture, peak broadening arising from the counter tube cannot be tolerated, and this can only be avoided by sacrificing the detection sensitivity Alternatively, in order to enhance the

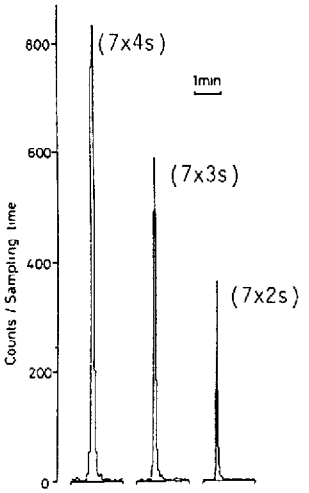


Fig 2 Relationship between the maintenance of chromatographic resolution and the improvement of detection sensitivity [7] Sample [^{14}C]hexadecane, ca 56 Bq Figures in parentheses are residence times 1 m×3 mm I D glass column packed with OV-1 on Shimalite W (80–100 mesh), 250°C

overall sensitivity, the RT must be made larger This can be achieved by using a counter tube of larger volume and/or decreasing the total gas flow-rate Reeve and Crozier [16] recommended utilizing a cell for RHPLC with a volume one third of the peak volume of the narrowest chromatographic peak of interest They also mentioned that under these circumstances high sensitivities are obtained with no more than a 10% increase in peak width Sieswerda et al [17], Reich et al [18] and Klein and Hunt [19] treated this problem more mathematically Similar considerations seem to be true for RGC RT values in most RGC systems are between 10 and 20 s, with few being beyond this range (see Table 1)

Another important problem in RGC is peak distortion or increased tailing, which is caused by various factors The first factor may be the back-diffusion occurring in the gas passing through the splitter, oxidation–reduction tube and counter tube As will be mentioned later, however, the peak broadening that arises on passing through one counter tube of inner volume 10 ml was clarified as being not so large (less than 2% in half-width, see Section 4 3) With [^{14}C]hexadecane, distinguishable tailings in the RAD trace were not observed in comparison with the FID trace under any conditions (see Fig 15) These experimental results suggest that back-diffusion is not the main cause of tailing, unless the gas flow-rate is extremely low The other factors are the adsorption and/or condensation of radioactive components on the inside wall of the transfer line and the deterioration of the catalyst in the oxidation–reduction tube Therefore, the transfer line must be maintained at a temperature

higher than that of the GC oven Recent experience in our laboratory has demonstrated that even a slight deterioration of the catalyst in the oxidation–reduction tube results in appreciable tailing of the RAD trace with a capillary column Therefore, it is advisable to renew the catalyst before the total amount of injected solvent reaches a certain level, e g , 50 μl in our case

Many authors compare ambiguously the chromatographic resolution of an RAD trace with that of an FID trace To make the matter simple, suppose the factors causing peak distortion are excluded and the radiochromatograms are being recorded with a reasonably short time constant To the first approximation, the radioactive peak may be considered to suffer from peak broadening with time corresponding to twice the RT (or twice the sampling time with digital recording) at the most, independent of the peak width of the FID trace [19] The validity of this conclusion is evident by examination of the radiochromatograms shown later in Figs 3–5, 15 and 17, where FID traces are presented together with RAD traces

Many workers calculate the counting efficiency (E) from the following equation·

$$E\ (\%) = \frac{\text{observed counts} \cdot 100}{\text{applied radioactivity (Bq)} \cdot \text{RT (s)}}$$

Kiricsi et al [9] mentioned that the counting efficiency is significantly affected by the composition of gas passing through the counter tube but not by the width of the chromatographic peak

The reported counting efficiencies of gas flow proportional counters for ^{14}C are around 90%, whereas those for ^{3}H vary widely, as shown in Table 1 In the application of this equation, however, we must consider seriously the fact that not all the applied radioactivity may reach the RAD system even when the effluents are led to the detector without splitting One reason is the loss due to the adsorption, condensation and thermal decomposition of radioactive components on the column and the inside wall of the injection port and transfer line, the other is incomplete combustion The actual counting efficiency (i e , counts/radioactive disintegrations) can be considered to be nearly constant, independent of the chemical forms being radioassayed Although the oxidative decomposition process itself proceeds almost quantitatively [5], the conversion ratio of $^{3}H_2O$ to $^{3}H_2$ may vary, depending on the reducing conditions This seems to be the main reason why the reported counting efficiencies for ^{3}H vary so much Therefore, it is advisable to correct for the loss mentioned above and the conversion ratio (for ^{3}H) by using [^{14}C]- or [^{3}H]hexadecane as standards, which can be considered not to suffer from the loss to a significant extent

The background counts depend on both the type of RAD and the extent of shielding In radiotracer experiments, the detection limit is usually defined as the amount of radioactive substance giving radio signals three times the stan-

dard deviation of the background counts. Therefore, the background counts have to be lowered as far as possible. The background counts of gas flow proportional counters of 10 ml volume are usually around 30 cpm. Simpson [11] decreased the background counts considerably by use of γ-ray shielding and an anti-coincidence circuit between a gas flow proportional counter (for GC effluents) and a plastic phosphor guard counter.

The detection sensitivity in RGC is influenced by the chromatographic characteristics of the peak of interest (peak shape and GC peak yield [5]), the splitting ratio (RAD/mass detector), the counting characteristics (counting efficiency and background counts) and RT (for continuous methods) or the fractionation interval and counting time (for discontinuous methods). Therefore, it is meaningless to discuss the detection limit without taking all these factors into consideration. Roughly, the detection limit in RGC is several becquerels per peak without splitting to the mass detector, and radioactivity ten times this value is needed for quantitation

3 RECENT PROGRESS IN RADIO-GAS CHROMATOGRAPHY

3 1 Introduction of capillary columns

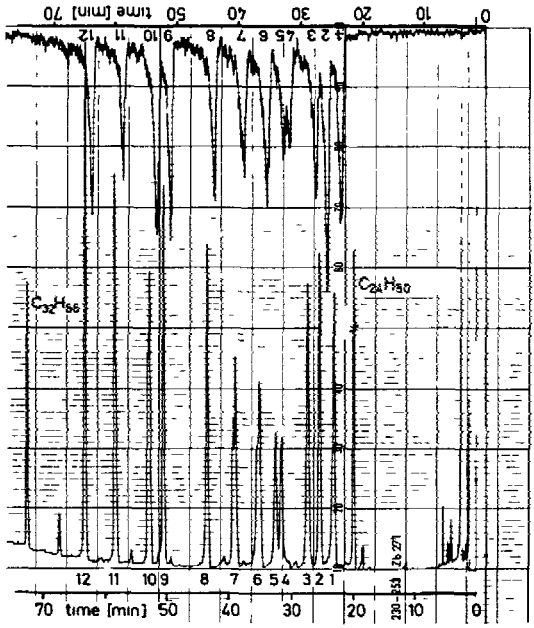
The incorporation of fused-silica capillary columns into GC analyses (in 1979) rapidly extended the applications of GC owing to the increased theoretical plate counts and the inertness of the inner surface of the columns. Several papers [6, 12–14, 20–22] were published on RGC systems with capillary columns It is a matter of great concern how far RAD can follow the chromatographic resolution of an FID trace. In order to maintain the high chromatographic resolving power of capillary columns, the RAD and/or the detecting conditions should be improved. However, the same detectors and conditions as applied with RGC system with packed columns are usually transferred to those with capillary columns. Consequently, the improvements in radioactive peak resolution are attributable only to the column resolving power In this instance, the compromise between the chromatographic resolution and detection sensitivity should be shifted to the former, and the experiments should be so designed as to make the RT a few seconds

Herkner [13] developed an RGC system with fused-silica capillary columns The system is based on a dual-column gas chromatograph equipped with a column-switching facility and a variable splitter at the column outlet combined with a dead-volume-free adapter for RAD The first column works as a separation column and the second is used as a feed to the mass detector The adjustment of the splitting ratio is regulated by the inlet pressures of the carrier gas supplying both columns. The radioactivity in the GC effluents is monitored by a gas flow proportional counter after decomposition Three flow modes can

be selected· (a) all the column effluents to the mass detector or (b) to the RAD and (c) simultaneous flow to the mass detector and RAD. Fig 3 shows a simultaneous mass and radiochromatogram, in which a test mixture consisting of eleven steroids was separated as methyloxime–trimethylsilyl (MO–TMS) derivatives The radioactivity of the components is between 53 and 80 Bq each and the amount of unlabelled steroids 50 ng per component The mass detector/RAD splitting ratio is 3 1. The RT was calculated to be 6 s. The detection limit under these conditions was reported to be 13 Bq It was mentioned that the peaks in the RAD trace showed nearly the same shapes as those in the mass detector trace.

Ernst et al [12] constructed a capillary RGC system equipped with a commercial gas flow proportional counter, and compared the performance of packed and capillary columns with a sample of 'ene-ol standard'. They mentioned that the increased resolution inherent in the capillary column was realized and the detection limit was 1 7 Bq with their system The RT calculated from the data given in the experimental section is 20 s

Rodriguez et al. [6] described an improved gas flow proportional counter tube of inner volume ca 5 ml for fused-silica capillary columns, in which the



Steroid	Number
3α 17β-dihydroxyandrost 5α-ane	1
3α-hydroxyandrost 5α-ane-17-one	2
3β-hydroxyandrost-5 ene-17-one	3
17β-hydroxyandrost-5α-ane 3-one (5)	4 5
17β-hydroxyandrost-4-ene 3-one	6
Androst-4-ene-3 17-dione	7
3β-hydroxypregn 5-ene-20-one	8
Pregn-4 ene-3 20-dione	9
17α hydroxypregn-4 ene-3 20-dione	10
3β 17α-dihydroxypregn-5-ene-20-one	11
3β hydroxycholest-5-ene	12

Fig 3 Radio-gas chromatogram of test mixture consisting of eleven [^{3}H]steroids (as MO–TMS derivatives) [13] Top, RAD trace, bottom, FID trace 25 m×0 2 mm I D fused-silica column coated with CP-SIL 5 (0 12 μm), column temperature, programmed from 200 to 263 °C at 1 5 °C/min (Reproduced with permission)

radioactivity per 6 s is recorded as a histogram They pointed out that deterioration of the catalyst in the combustion tube causes a back-pressure and residual radioactivity, the former results in severe fluctuation of the splitting ratio and the latter in tailing of the radioactive peaks They recommended copper oxide wire mixed with quartz particles as the tube packing and continuous introduction of a minute amount of oxygen into the tube The chromatographic performance of their system is illustrated in Figs 4 and 5. In this system the radioactive peaks are 12 s wide (i e , corresponding to two sampling times) and the detection limit for ^{14}C is 7.4 Bq ($RT \approx 4$ s)

Gross et al [20] reported a capillary GC system coupled with an anthracene flow cell. They minimized the dead volumes of the splitter (10 μl) and combustion tube (100 μl) as far as possible to maintain the resolving power of the capillary columns. The combustion tube is open to the atmosphere along the inlet capillary A suction pump connected to the flow cell furnishes a negative pressure Pressure waves generated by combustion in the furnace are prevented from being transferred back into the GC system by these two devices. The peak resolution of their ^{14}C detection system under practical conditions was about half that of FID and the detection limit for ^{14}C was 7 Bq

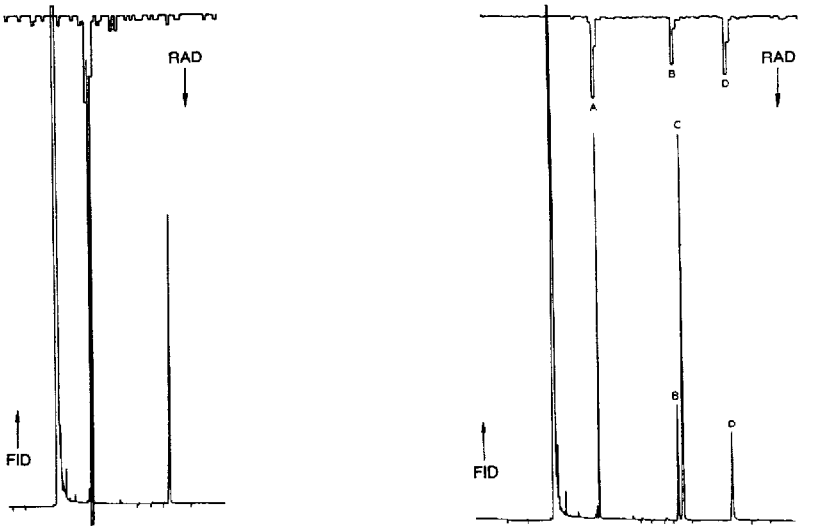


Fig 4 Chromatogram of n-$^{14}C_{10}$ (elutes first) and n-C_{12} hydrocarbons [6] Column, 30 m×0 32 mm I D Durabond 5J&W column, temperature programme, 110°C, held 4 min, increased at 4°C/min to 120°C and held 100 counts full-scale RAD/FID splitting ratio, 46 54 Mass injected ca 70 ng per compound (Reproduced with permission)

Fig 5 Chromatogram of ^{14}C-labelled standard materials and cold n-C_{12} [6] Mass (radioactivity) injected A, n-$^{14}C_{10}$, 63 ng (89 Bq), B, [^{14}C]naphthalene, 25 ng (33 Bq), C, n-C_{12}, 110 ng, D, n-$^{14}C_{10}OH$, 38 ng (56 Bq) GC conditions as in Fig 4 (Reproduced with permission)

3 2 Discontinuous methods

Wels [23] reported a discontinuous method which involves combustion of components by use of FID and trapping the $^{14}CO_2$ and/or $^{3}H_2O$ in an absorption fluid which, after addition of a scintillation fluid, is collected in fractions and counted in a liquid scintillation counter The absorption cell, as shown in Fig 6, is constructed entirely from glass It consists of four concentric tubes The innermost tube (A) carries the gases from the flame ionization detector into the cell It has a jacket tube (B) and the space between the tubes A and B is heated by a resistance wire to prevent condensation of $^{3}H_2O$ inside tube A The outermost tube (D) acts as a condensation–absorption surface for $^{3}H_2O$ and $^{14}CO_2$, and during operation the inner surface of this tube is constantly flushed by an even film of absorption fluid 2-Methoxyethanol (2-ME) or 2-ME containing 10% of 2-phenylethylamine was used as the absorption fluid

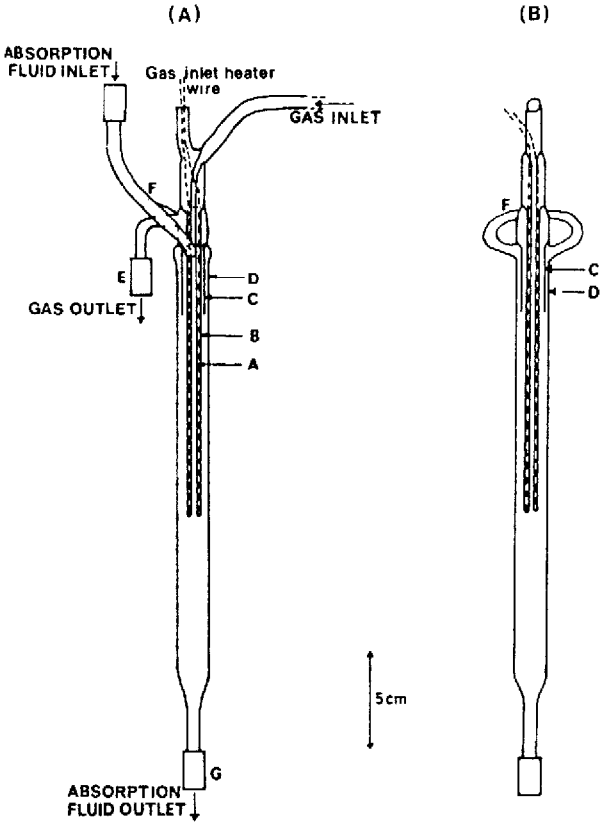


Fig 6 Descending film absorption cell [23] (A) Front view (B) view from right-hand side Dimensions tube A, 4 mm O D , 2 3 mm I D , tube B, 6 5 mm I D , tube C, 13 mm O D , tube D, 14 mm I D (Reproduced with permission)

for $^{3}H_2O$ or $^{14}CO_2$, respectively A typical radio-gas chromatogram is shown in Fig 7, in which amino acids were separated in 20-s fractions as their N-trifluoroacetyl [^{3}H-methyl]methyl ester derivatives There is little loss of resolution apart from that involved in the taking of fractions The latter point mainly accounts for the lack of resolution of the glycine and alanine peaks The overall collection efficiency for a chromatographic run (i e , from collection to injection) was 92% It was suspected that the major loss of radioactivity might be due to adsorption on the GC column, and it was mentioned that if the

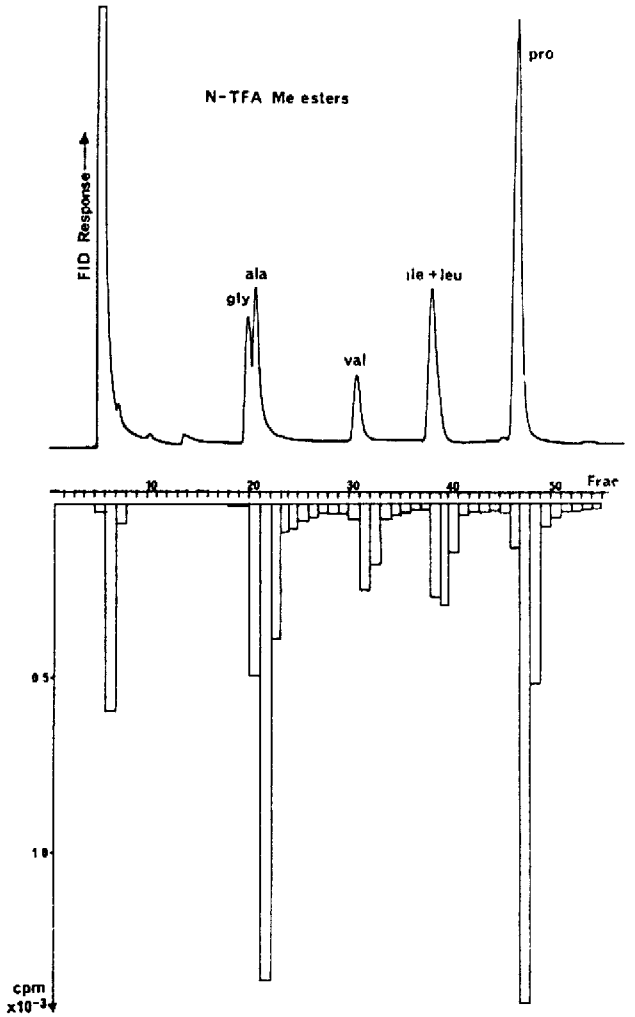


Fig 7 Radio-gas chromatogram of amino acids as their N-trifluoroacetyl [^{3}H-methyl]methyl esters (5 μg, 350 Bq) [23] Column, 274 cm×0 2 cm I D glass column packed with 5% OV-1, temperature programmed from 75 to 130 °C at 6 °C/min (Reproduced with permission)

radioactivity from a single peak was collected in two or three fractions, then radioactivity as low as 1 7 Bq in that peak could be detected (10-min counting time per fraction) Regarding dual-label RGC, chemical separation was found to be preferable to differentiation using a liquid scintillation counter because of the difficulties associated with dual-label counting.

In discontinuous methods, the resolving capability is strictly limited by the time interval of fractionation To maintain the peak resolution, this interval should be set to a suitable period Hamnett and Pratt [21] used capillary column RGC in combination with a discontinuous method similar to that of Wels [23] for the identification of biosynthesized insect hormones One example of their radio-gas chromatograms is shown in Fig 8 Here, the biosynthesized [^{3}H-methyl]methyl(2*E*,6*E*) farnesoate is clearly separated from its geometric isomer. They collected automatically fractions of effluent from the absorption cell every 12 s This is the reason why the peak resolution in the radiochromatogram is much improved in comparison with that shown in Fig 7 Lee et al [24] reported a simple method for trapping ^3H_2O using a disposable glass pipette (Corning, 13 cm×0 5 mm I D)

Derks et al [25] developed another discontinuous method, in which a gas chromatograph is fitted with a splitter leading the column effluents to a heated all-glass outlet The outer end of the glass outlet is bent downwards and posi-

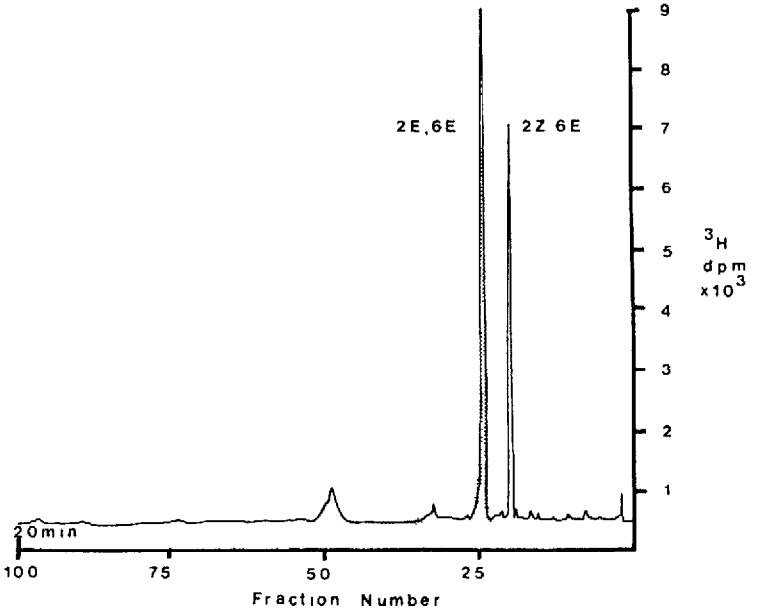


Fig 8 Radio-gas chromatogram of the methyl farnesoate (MF) zone from TLC of the products of *Periplaneta americana* CA, stimulated with farnesenic acid and cultured with [^{3}H-methyl]-methionine [21] Non-radioactive geometric isomer of MF was added Column, 20-m Ucon HB5100 capillary column, 170°C (Reproduced with permission)

tioned as close as possible to the adsorbent layer of a thin-layer chromatographic (TLC) plate, which can be moved horizontally By moving the plate slowly during a GC run, the eluting radioactive components are trapped on the TLC plate separately. The adsorbent in the zones on the plate corresponding to the positions of radioactive components are scraped off and counted in a liquid scintillation counter

3 3 Others

Saito et al [26] proposed a long-path gas flow proportional counter as a high-sensitivity detector for RGC The counter tube for general use is made of brass or copper (100 cm × 1 cm diameter), with a mirror finish on all the internal surface, and the centre electrode is a tungsten wire 0 05 mm in diameter For actual use, several of these counter tubes are connected in series to hold the total volume of a gas sample in the counting system over a desired counting period The radioactivity is recorded as integral counts with good retention of chromatographic resolution, as the counter tube has a sufficiently long passage in comparison with the diameter The counting efficiencies, which were determined by direct injection of a known amount of [^{3}H]- and [^{14}C]toluene, were 93 and 89%, respectively

High-temperature applications of RGC exist that require non-destructive continuous analyses of GC effluents because the effluents must be trapped for further study Gordon et al [27] developed a gas flow proportional counter for continuous operation at 300 °C The problem of loss of the proportional counting plateau at higher temperatures was solved by carefully honing and electropolishing the flow counter barrel and using a centre wire free of defects. In this system, the background counts are low (ca 75 cpm) and the counter is decontaminated in situ by treatment with oxygen at 325–350 °C This system can be used for the reactions of accelerated carbon ions impinging on various targets, primarily benzene Netting and Barr [28] also designed a gas flow proportional counter at elevated temperatures for RGC, in which the methyl esters of higher fatty acids could be collected from the effluents with an efficiency of about 40% relative to the amount injected.

Two papers on isotope effects on GC retention times were published Berger et al [29] described a method for separating the simple radioactive precursor $^{11}CH_4$ from its natural carrier $^{12}CH_4$ by capillary GC at very low temperatures on hydrated, soft-glass columns, using helium–nitrogen mixtures as the eluent They obtained $^{11}CH_4$ with an extremely high specific radioactivity (0 74–11.1 TBq/μmol) by this method Gordon et al [30] reported a small reduction in the GC retention times of a number of tritiated compounds compared with their unlabelled analogues. The shift appears to be proportional to the number of tritium atoms in a molecule

4 RADIO-GAS CHROMATOGRAPH EQUIPPED WITH A SYNCHRONIZED ACCUMULATING RADIOISOTOPE DETECTOR

In conventional GC systems, the column effluents are monitored by a single detector, irrespective of RAD or mass detectors, without exception It must be mentioned that the RGC system with a single counter tube does not permit a simultaneous improvement in both the detection sensitivity for radioactivity and the chromatographic resolution, as discussed in Section 2 2 We have developed a SARD which can limit this drawback substantially

4 1 Operating principle

It should be possible to obtain a high detection sensitivity without sacrificing chromatographic resolution, when n small counter tubes of equal volume are connected longitudinally and the radioactivity signals detected by each counter tube are accumulated in synchronization with the travelling speed of the radioactive components On the basis of this fundamental principle, we developed SARD, which is applicable to various radiochromatographs. Fig. 9 illustrates the principle of SARD for $n=3$ Suppose a sample travelling through the counter tubes C_1, C_2 and C_3 at a flow-rate u ml/s, and the volume of each counter tube being v ml Here, a s is defined as the sampling time, given by v/u RT in SARD is defined as the time required for a specified point in the sample to pass through the whole detector assembly, the value being given by na. The idea is to accumulate the radioactivity signals toward the direction indicated by the bold arrows into an accumulation counter

A block diagram of a SARD composed of three counter tubes is shown in Fig 10 It consists of three parts, i e , a radiodetector assembly, switching units (B) and accumulation counters (A). The radiodetector assembly consists of a series of small counter tubes (C) of equal volume which are connected longitudinally Each switching unit has one output and three input terminals A contact arm connects the output terminal with one of the input terminals The radioactivity signals of a point in the effluent are accumulated in the specified

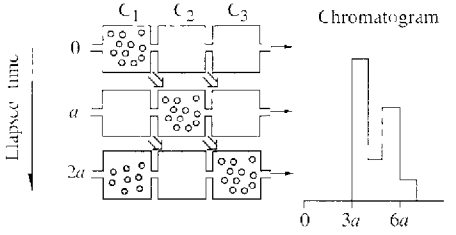


Fig 9 Elapsed time and positions of radioactive components in the SARD detector assembly composed of three gas flow proportional counter tubes C_1–C_3, counter tubes, a, sampling time

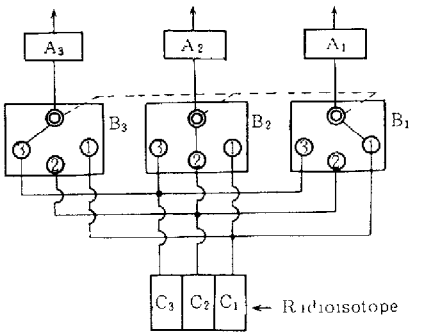


Fig 10 Mechanism of synchronized accumulation of radioactivity signals in the SARD composed of three counter tubes C_1-C_3=counter tubes, B_1-B_3=switching units, A_1-A_3=accumulation counters, ◎, output terminals, ○, input terminals

accumulation counter (or one of the specified two adjacent accumulation counters, see Fig 11) at any time by means of the switching unit When their detection by the third counter tube is completed, the accumulated signals are sent to a recorder, and the counting cycle is reset to the initial state It is not necessary to ensure uniformity of the background counts and counting efficiencies in each counter tube, as all portions are treated equally by all the counter tubes

The movement of the contact arm in each switching unit can be synchronized with the travelling speed of the radioactive components The radioactivity signals of the first portion, which are detected by C_1, C_2 and C_3 during the time intervals 0–a, a–2a and 2a–3a, respectively, are sent to the accumulation counter, A_1, through the input terminals 1, 2 and 3 of B_1 The accumulated signals are then sent to a recorder and recorded as the radioactivity of fraction 1 The same procedures are followed for the next portion entering the detector assembly, the radioactivity signals being accumulated in A_3, and recorded as the radioactivity of fraction 2

The counting conditions in SARD are shown schematically in Fig. 11 The dots indicate the time point for a switchover of the contact arm in each switching unit The radioactivity signals at the points marked with the dots are accumulated at any time in the specified accumulation counter and recorded as the radioactivity of the corresponding fraction. For example, the radioactivity signals at point X in the effluent, which enters the detector assembly at just one sampling time after the beginning of detection of the seventh portion by C_1, are fully accumulated in A_1 and recorded as the radioactivity of fraction 7 On the other hand, the radioactivity signals at the other points are split between the specified two adjacent accumulation counters. For example, the radioactivity signals at point Y, which enters the detector assembly at a time 1 6a after the beginning of detection of the third portion by C_1, are split between A_2

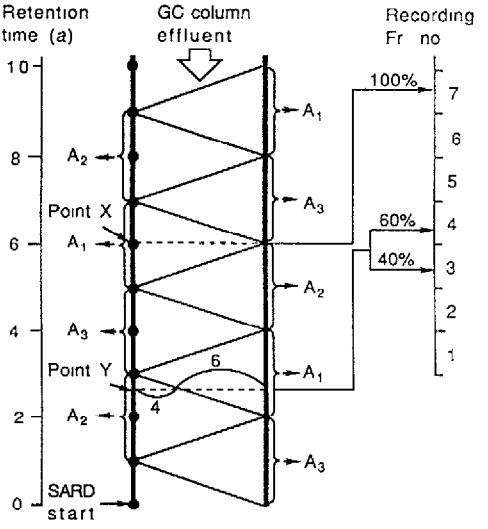


Fig 11 Counting conditions of the SARD composed of three counter tubes A_1-A_3 = accumulation counters

and A_1, and 40% are recorded as the radioactivity of fraction 3 and 60% as that of fraction 4

4 2 Instrument and operating conditions

The feasibility of SARD was first verified for detection in TLC [31] and then in HPLC [32,33] In 1980, we published the first report on an RGC system equipped with SARD , composed of five gas flow proportional counter tubes of inner volume 30 ml [10] Six years later, we developed an improved SARD for RGC, in which the five counter tubes are connected by a mutual anti-coincidence circuit [34] With this improvement the background counts were approximately halved. The detection limit of the improved SARD for [^{14}C]hexadecane was approximately 0.74 Bq as amount injected. Recently, we designed a new SARD system composed of seven gas flow proportional counter tubes of inner volume 10 ml that can be applied to both capillary and packed columns [7,35] The data given below are those obtained with the new SARD, unless stated otherwise

Fig. 12 shows a block diagram of an RGC equipped with the new SARD [35], which was manufactured by Aloka (Murei, Mitaka, Tokyo, Japan) The seven gas flow proportional counter tubes (I D , 10 mm; effective length of anode wire, 128 mm) are arranged in the closest packed structure, and placed in a 30-mm-thick cylindrical lead housing The counter tubes are electrically connected by a mutual anti-coincidence circuit

The SARD system can be connected to either packed glass columns or wide-

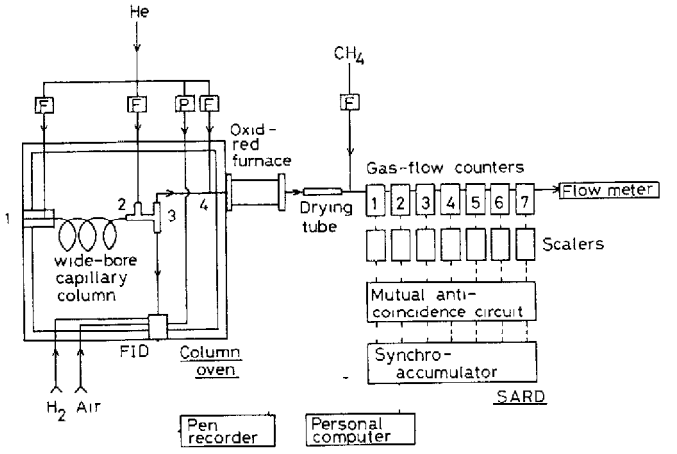


Fig 12 Schematic diagram of RGC system on wide-bore capillary column [35] 1 = Injection port (direct injection), 2,4 = addition of make-up gas, 3 = column effluent splitter, F = constant-flow regulator, P = constant-pressure regulator

TABLE 2

COUNTING CONDITIONS

Counting condition	Flow-rate (ml/min)		Sampling time (s)	High voltage (kV)	Peak broadening[a]
	Helium	Methane			
A	50	100	4	2 78	1 29 ± 0 064
B	50	150	3	2 95	1 13 ± 0 037
C	50	250	2	3 06	1 10 ± 0 040
D	50	550	1	3 20	1 15 ± 0 076

[a]Ratio of the half-width values (seventh counter tube/first counter tube)

bore fused-silica capillary columns The most distinctive feature is that the RT is variable between 7 × 1 and 7 × 4 s by regulating the flow-rate of the counting gas (methane) Because the plateau characteristics of gas flow proportional counters vary with the gas composition, the high voltage of the radiodetector assembly is set at the optimum value Table 2 summarizes the measurement conditions Neither the background counts nor the counting efficiency are affected by the gas composition to a significant extent

In the RGC–SARD system, the fraction number, the counts per fraction and the integral counts are printed simultaneously with the recording of the radiochromatogram The radioactive peak intensity can be easily calculated by subtracting the integral counts of the fraction corresponding to the beginning of the peak from those to the end of the peak

4 3 Performance

4 3 1 Feasibility

The usefulness of SARD for RGC is largely dependent on how much peak broadening occurs owing to the prolonged RT of the radioactive components in the multiple counter tubes This was examined by injecting 150 Bq of [^{14}C]hexadecane and monitoring the radioactivity simultaneously with the first and seventh counter tubes of SARD under the conditions A–D in Table 2 The peak broadenings judged from the increase in half-width values were relatively small and approximately constant (1 10–1 15) when the total gas flow-rate was more than 200 ml/min (under conditions B, C and D) In contrast, the value was slightly larger at a total gas flow-rate of 150 ml/min (under condition A) However, the peak broadening observed under this condition does not seem to impede the applicability of SARD, as will be discussed later (see Figs 14 and 15) An interesting finding was that the peak broadening accompanied by passing through one counter tube, for example at a total gas flow-rate of 200 ml/min, could be estimated to be 1 8%, as follows: when the peak broadening is set at x, then $(1+x)^7=1.13$ and $x=0\ 018$

[^{14}C]Hexadecane was injected into the RGC–SARD system and the radioactivity was measured simultaneously by both the conventional methods and SARD under condition C in Table 2. An example of the chromatograms and reproducibility data are shown in Fig 13 The chromatogram in I is the output signal from the first of the seven counter tubes constituting the SARD system The chromatogram in II is the sum of output signals from all seven counter

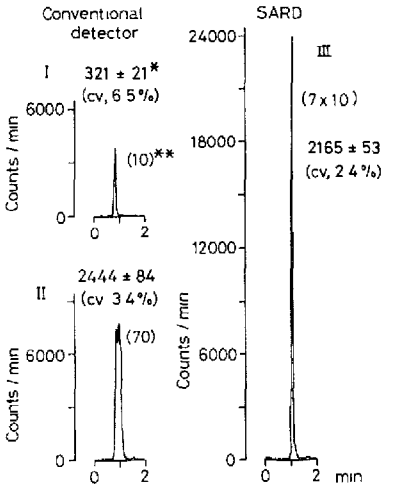


Fig 13 Comparison of chromatogram obtained with SARD with those from conventional detection methods [7] Sample [^{14}C]hexadecane, ca 150 Bq * = Counts (mean ± S D), ** = detector volume (ml) GC column as in Fig 2, 180 °C Counting condition, C in Table 2

tubes, and may be considered to result from a single counter tube of volume 70 ml The chromatogram in III is the SARD output signal The advantages of SARD are evident by comparison of the three chromatograms The resolution is sacrificed in II to give extreme peak broadening. However, the peak width in III is almost identical with that in I As is shown from the reproducibility data, the integral intensity in III is comparable to that in II and about seven times that in I Although the coefficient of variation in II is improved by $1/\sqrt{7}$ times that in I, that in III obviously improved even more Whereas the peak intensities in I and II are calculated from the areas which are measured by cutting and weighing the radioactive peaks, those in III are obtained directly from the chromatograms and the corresponding improvement in precision is achieved The above results show that both the maintenance of chromatographic resolution as in I and the enhancement of detection sensitivity as in II are realized simultaneously in SARD, whereas choice must be made in the conventional method

4 3 2 Background counts

Reduction of the background counts also contributes to the precise detection of peaks of low radioactivity An effective low-background detector was designed by Simpson [11], as stated above An attempt was made to develop a low-background SARD by a mutual anti-coincidence method [34] In the usual anti-coincidence method, the main counter tube is surrounded by the guard counter tubes, and pulses from the main counter tube (main pulse) are controlled by those from the guard counter tubes (gate pulse), that is, the gate pulse determines whether the main pulse is counted or not In the mutual anti-coincidence method, pulses from each counter tube serve as both main and gate pulses The background counts in the new SARD were approximately 40 cpm, i e , 6 cpm per counter tube This value is considered to be fairly low compared with those in usual gas flow counter tubes having the same volume [^{3}H]Hexadecane in an amount from 20 to 180 Bq was injected into the RGC–SARD system to examine the linearity between the injection radioactivity and the radioactive peak intensity An excellent linear relationship was found The amount of radioactivity injected into the RGC is normally less than 180 Bq per component in biochemical studies Therefore, the counting loss due to an accidental coincidence of the radioactive disintegrations in the detector assembly was proved to be negligible in practical samples

4 3 3 Detection limit and accuracy of quantitation

In order to examine the detection limit, a series of samples containing [5,6,8,9,11,12,14,15(n)-^{3}H]arachidonic acid ([^{3}H]AA, 7 1 TBq/mmol) of relatively low radioactivity were measured as the methyl ester under condition A in Table 2 The results are given in Fig 14 [36]

Although the detection limit of the conventional method is determined more or less subjectively, that of the present method may be calculated more objec-

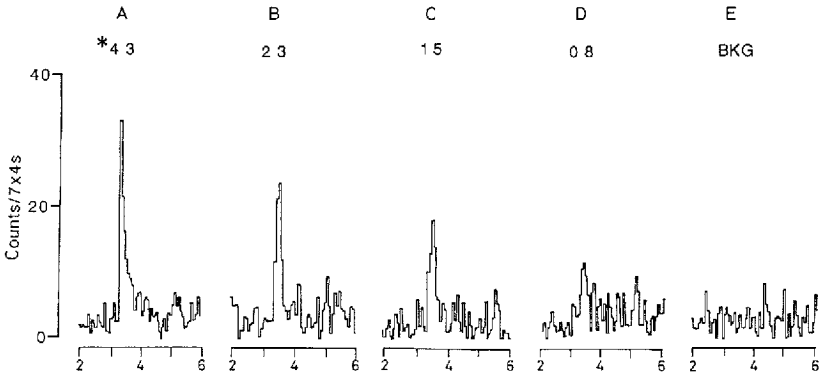


Fig 14 Detection limits Sample [^{3}H]arachidonic acid (7 1 TBq/mmol) [36] * = Injection radioactivity (Bq) CBP-1 wide-bore fused-silica capillary column, 12 m × 0 53 mm I D , film thickness 1 μm Column temperature programmed from 210 to 290 °C at 20 °C/min BKG = background Counting condition, A in Table 2

tively and treated as a matter of probability For the conventional method the detection limit is usually discussed on the basis of the signal-to-noise ratio (S/N), and S/N = 3 is usually used as the detection limit The detection limit of SARD is more reasonably discussed on the basis of the total counts of several successive fractions, not the counts of one fraction, as the radioactivity in a peak appears over several fractions We proposed the following parameters to define the detection limit for SARD [34] $TCSF_n$ = total counts of n successive fractions, $TCSF_{n\,back} = TCSF_n$ in the background region, $TCSF_{n,max} = TCSF_n$ which gives the highest value in the region of the peak of interest (corrected for the background counts) Here n is the number of fractions which make $TCSF_{n,max}$ at least 90% of the total counts of the peak of interest The detection limit for SARD may be defined as the radioactivity injected (x Bq) giving $TCSF_{n\,max}$ exceeding 3 (S D. of $TCSF_{n,back}$) and calculated as follows

$$3(\text{S D of } TCSF_{n,back}) = x \times \text{GC peak yield} \times \text{oxidation–reduction efficiency} \times \text{counting efficiency} \times \text{RT (s)} \times TCSF_{n,max}/\text{total counts of the peak}$$

For example, the detection limit of [^{3}H]AA calculated from this equation was 1 1 Bq (ca 50 fg)

As the radioactive disintegration suffers from a statistical fluctuation, two probabilities should be considered when discussing the detection limit One is the probability (α) that $TCSF_{n,back}$ exceeds the detection limit, giving a false peak The other is the probability (β) that $TCSF_{n\,max}$ of a sample containing a certain amount of radioactivity is below the detection limit, giving no peak When 3 (S D of $TCSF_{n,back}$) is the detection limit $\alpha = 0\,14\%$ The detection limit calculated above is a value that can be characterized as a peak with detection level ($100 - \beta$) of 50% This detection limit is much superior to those

attained with the same chromatographic resolution (i e , with an RT of 4 s) in the conventional system However, this is not the overestimated value at all, as shown in Fig. 14. ^{3}H-Labelling usually gives labelled compounds of an extremely high specific activity Therefore, the combination of this RGC–SARD system with the use of ^{3}H-labelled compounds as a tracer is expected to provide a powerful technique having a high sensitivity comparable or even superior to that of selected ion monitoring in GC–MS

The accuracy of quantitation is dependent on the amount of radioactivity injected The coefficients of variation when 11 1 and 118 Bq of [^{3}H]androstenedione were injected were ca 12 and 3%, respectively [35]

4 3 4 Compromise between chromatographic resolution and detection sensitivity

As mentioned above, the most distinctive feature of the new RGC–SARD system is that a compromise between the chromatographic resolution and detection sensitivity is made very easily by regulating the flow-rate of the counting gas [35] The chromatograms obtained under conditions A (sampling time 4 s), C (2 s) and D (1 s) in Table 2 are shown in Fig 15 With the decrease in sampling time, the peak width of the RAD trace becomes narrow, approaching to that of the FID trace When detection sensitivity is to be preferred, condition A is recommended The RAD traces C and D have substantially the same

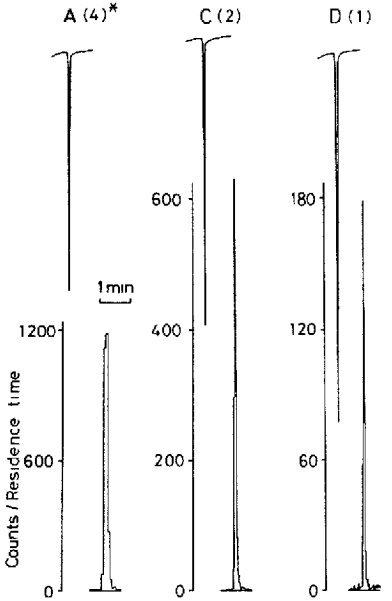


Fig 15 Influence of sampling time on chromatographic resolution [35] Sample [^{14}C]hexadecane, ca 100 Bq * = Sampling time (s) A, C and D correspond to A, C and D in Table 2, respectively Top, FID trace, bottom, RAD trace GC column as in Fig 14 Column temperature programmed from 100 to 220 °C at 40 °C/min

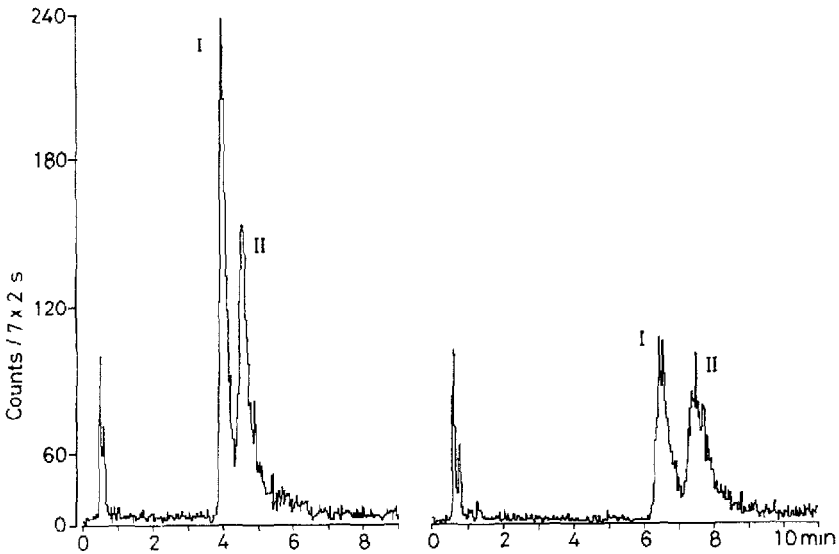


Fig 16 Radio-gas chromatograms produced by capillary RGC–SARD (left) and packed RGC–SARD (right) [35] Sample 190 Bq of *dl*-[^{14}C]suprofen-(*S*)-(−)-1-(1-naphthyl)ethylamine Counting condition, C in Table 2 GC column as in Fig 14 Peaks I = *d*-suprofen, II = *l*-suprofen

chromatographic characteristics as the FID trace Therefore, when high resolution is required, condition C should be chosen

An example in which the advantages of the new RGC–SARD system on a capillary column are well realized is shown in Fig 16 The sample is *dl*-[^{14}C]suprofen-(*S*)-(−)-1-(1-naphthyl)ethylamine The oven temperatures were identical in both runs shown It can be assumed that the superiority of the GC peak yields and the precision in the capillary column are due to the suppression of column adsorption and thermal decomposition of the substances due to the inertness of the column and the short time required for analyses [35]

5 APPLICATIONS

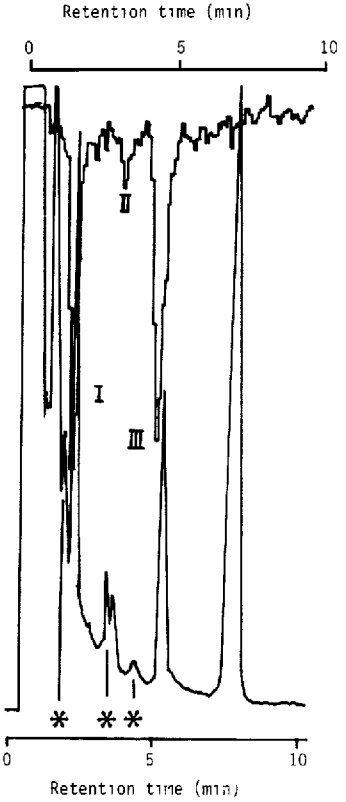
RGC is a mature technique, on which many chromatographers formerly placed high hopes However, today RGC seems to play a subordinate role in chromatography Only a few papers have been published in which the term 'radio-gas chromatography' is mentioned Therefore, it is impossible to write a comprehensive review about its applications In the following, representative studies in which RGC was used efficiently are mentioned

5 1 Metabolic studies

RGC is a useful tool for searching for metabolites originating from a radioisotopically labelled compound in a complex matrix Recently, stable isotope tracer techniques have been enjoying a broad application in drug metabolic

studies in combination with the development of GC–MS [37,38] One of the weak points of the tracer techniques is that stable isotopes are 'silent' isotopes. Hence RGC is expected to become a technique complementary to these newly developed tracer techniques

2-[4-(2-Thienyl)phenyl]propionic acid (suprofen) is a non-steroidal anti-inflammatory drug Its metabolic pathway was studied by RGC in combination with isotope ion cluster techniques [39]. TLC of the intact urine of rats given 10 mg/kg [2-^{14}C]suprofen revealed two groups of metabolites: a polar group (R_F 0 0) and a less polar group (R_F 0 4–0 8) A typical radio-gas chromatogram of the less polar group is shown in Fig. 17 Three radioactive metabolites with retention times of 1 5 (peak I), 3 3 (peak II) and 4 4 min (peak III) marked with asterisks were observed The less polar group of metabolites obtained from rat urine after oral dosing with 20 mg/kg of an equimolar mixture of suprofen and [phenyl-^{2}H$_4$]suprofen were analysed by GC–MS under the same



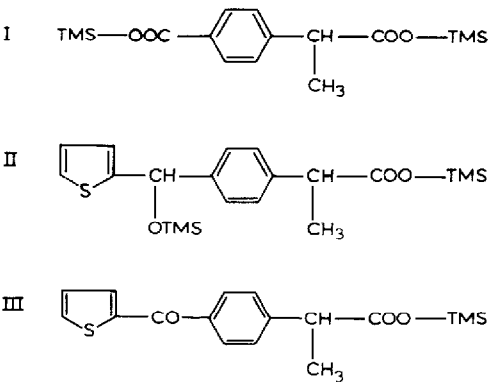


Fig 17 Radio-gas chromatogram of metabolites of [^{14}C]suprofen (rat urine, as trimethylsilyl derivatives) [39] Column, 2 m×3 mm I D Glass column packed with 1 5% OV-17, 225 °C (Reproduced with permission)

conditions as the RGC analysis Mass spectra of peaks corresponding to peaks I, II and III showed the expected doublet peaks 4 atomic mass units apart RGC was helpful in distinguishing metabolites separately from the solvent, derivatizing reagents and biological impurities, and the results were readily applicable to GC–MS techniques.

Another example of a metabolic study using RGC is shown in Fig 18 A 5-ng amount of [^{3}H]6-ketoprostaglandin $F_{1\alpha}$ (5 8 TBq/mmol) was administered intravenously to a rat The urine collection from 0 to 8 h was treated with Sep-Pak C_{18} and Sep-Pak silica and, after derivatization, the eluate was injected into the RGC–SARD system without any further purification [36] The RAD trace gave the well defined seven radioactive peaks, whereas the FID trace did not give any information, as predicted The radioactivity of each peak was calculated from peak counts, RT (7×4 s) and counting efficiency (65%), and expressed as a ratio to the radioactivity injected The mass spectra of the corresponding peaks of the urine of rat administered a large amount of 6-keto-dinor-7,9,13-prostaglandin $F_{1\alpha}$ (1 0 mg) suggested that peaks 1, 2 and 3 are dinor-4-keto-7,9,13-trihydroxyprosta-11,12-enoic acid, 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$ and 6-ketoprostaglandin $F_{1\alpha}$, respectively

These results demonstrate the three advantages of the RGC–SARD system The first is that the RAD trace does not suffer from any interference from endogenous components, which means that RGC analysis requires fewer pre-purification procedures and that there is less decomposition accompanying the

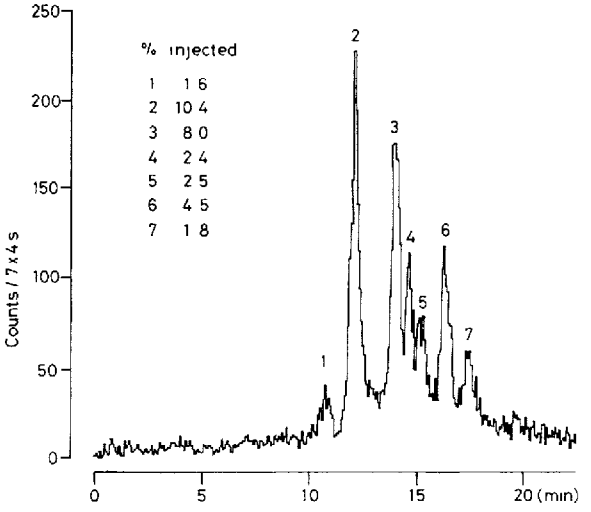


Fig 18 Radio-gas chromatogram of urinary [^{3}H]6-ketoprostaglandin $F_{1\alpha}$ and its metabolites (as methyl ester–methyloxime–dimethylisopropylsilyl derivative) Sample ca 310 Bq Counting condition, A in Table 2 GC column as in Fig 14 Temperature programme 240 °C, held 4 min, increased to 290 °C at 3 °C/min The broken line indicates the elevated background level

purification procedures The second is its extremely high sensitivity, which makes it possible to perform a metabolic study in vivo with nanogram dosages The third is that the data obtained by this system are much more easily accessible to quantitative analysis A 'balance sheet' can be made between the amounts of each metabolite recorded on the radiochromatogram and amounts injected, as mentioned above

A few reports in which RGC was used methodologically for metabolic studies of steroids have been published Lisboa et al [40] investigated the metabolism of [^{14}C]testosterone in the intact rat kidney, using a model in which the anatomical and physiological uninjured kidney was perfused Six labelled metabolites were isolated and identified by over-run TLC and RGC Weber et al [14] used RGC on capillary columns to detect trace amounts of estrogens and identified 7α-hydroxyestradiol-17β as a new metabolite of [^{14}C]estradiol-17β after perfusion of isolated rat brain

5 2 Clinical applications

Derks et al [25] described an RGC method for the determination of the rate of production of cortisol by measuring the isotope dilution of urinary cortisol metabolites Urine samples from eight adult patients who had received 37 kBq [^{3}H]cortisol orally were processed and injected into the GC system Butyloxime–trimethylsilyl derivatives of tetrahydrocortisol (THCL), *allo*-THCL and tetrahydrocortisone (THCE) were trapped separately on a TLC plate and counted in a liquid scintillation counter, as mentioned in Section 3 2 The method was calibrated by determining [^{3}H]THCL and [^{3}H]THCE of known specific activities The results were reasonably reproducible, the coefficients of variation ranging from 8 to 15% for *allo*-THCL and THCL and from 9 to 16% for THCE The correlation coefficients were excellent when the method was compared with a method involving TLC and spectrophotometry The most important advantage of RGC over TLC methods is that the cortisol metabolites are easily purified and measured in only one chromatographic step Further, the gas chromatogram shows the pattern of urinary steroid metabolites, therefore yielding additional information on adrenal cortical function

Herkner et al [41] proposed a unique method for the measurement of androgen 5α-reductase by RGC on capillary columns [^{3}H]Testosterone was incubated with foreskin tissue homogenates The specific radioactivities of 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol were determined by the simultaneous mass and radioactivity analysis of the steroidal fraction extracted from the incubation mixture Reaction rates were calculated from the specific radioactivities of these metabolites and the precursor

Loh et al [42] developed a novel method for the determination of nortriptyline in plasma After the nortriptyline had been extracted, it was acetylated

with [^{3}H]acetic anhydride and the amount of [^{3}H]acetylnortriptyline in the extract was determined by RGC. A linear calibration graph resulted from plotting peak areas against human plasma concentrations of nortriptyline ranging from 3 125 to 50.0 ng/ml. The method is more sensitive than TLC and GC methods that do not employ radioactivity and is capable of assaying 5 ng/ml nortriptyline in plasma The sensitivity of this radioacetylation method can probably be increased by using acetic anhydride of higher specific radioactivity.

5 3 Studies of biosynthesis

Møller [43] skilfully applied RGC for the identification of possible intermediates in the biosynthesis of cyanogenic glucosides L-[U-^{14}C]tyrosine was incubated with sorghum 'high-speed' pellet suspension in an NADPH regenerating system Aliquots of incubation mixture were lyophilized to dryness and then silylated. A silylated standard mixture containing approximately 8 μg of

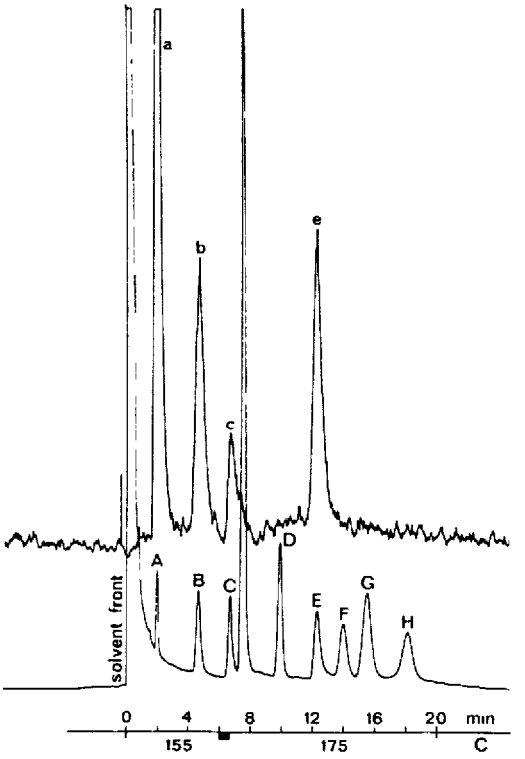


Fig 19 Radio-gas chromatogram obtained from an enzymatic experiment with sorghum microsomes fed L-[U-^{14}C]tyrosine [43] Top, RAD trace, bottom, FID trace Column, 168 cm×4 mm I D glass column packed with 3% SP-2250 Temperature programme, 155°C, held 6 min, increased at 30°C/min to 175°C, held 15 min (Reproduced with permission)

each of the potential intermediates was added as a carrier This allows the fast and accurate visual determination of the superimposability of GC–mass and radioactivity peaks. An example is shown in Fig 19. The mass peaks represent solvent front, *p*-hydroxybenzaldehyde (A), *p*-hydroxyphenylacetonitrile (B), *p*-hydroxyphenylacetaldoxime (C), tyramine (D), tyrosine (E), *p*-hydroxyphenylpyruvic acid oxime (F), N-hydroxytyrosine (G), *p*-hydroxyphenylpyruvic acid (H) and tricine (between C and D). The superimposable peaks representing labelled compounds formed on metabolism of L-[U-^{14}C]tyrosine are marked with the corresponding lower-case letters The method is especially advantageous in the determination of N-hydroxytyrosine, which is too unstable to be determined by more conventional methods such as TLC or paper chromatography.

6 CONCLUSIONS

RGC combines the advantages of radioisotope tracer techniques with those of GC RGC has not received much attention recently as GC has been largely replaced by HPLC during the last decade. However, RGC with a capillary column is expected to be a powerful tool for metabolic studies of radioisotopically labelled substances with high specific radioactivity. The SARD radioisotope detection system presented here makes it possible to improve the detection sensitivity without sacrificing the chromatographic resolution. Further, it should be pointed out that RGC is an excellent technique complementary to stable isotope tracer techniques, and is expected to undergo a renaissance in the near future

7 SUMMARY

Radio-gas chromatography (RGC) is a technique that combines the advantages of radioisotope tracer techniques with those of gas chromatography The constitution and performance of RGC systems in common use are described The relationships between the chromatographic resolution and detection sensitivity are discussed in a simplified form Recent developments in RGC systems during the last decade are reviewed A new detector system, named synchronized accumulating radioisotope detector, which makes it possible to improve the detection sensitivity without decreasing the chromatographic resolution, is discussed Applications of RGC in the life sciences are briefly presented

8 ACKNOWLEDGEMENT

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