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A TWO-DIMENSIONAL SCANNER FOR RADIOCHROMATOGRAMS EMPLOYING AN ELECTRON MULTIPLIER DETECTOR *IN VACUO*

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SUMMARY

A step scanner for the measurement of both one- and two-dimensional distributions of radionuclides in paper or thin-layer chromatography has been constructed and tested. Different versions of a miniaturized channel electron multiplier have been applied as detectors. A description is given of the principle of operation for this detector type, the vacuum scanner, and the way of data presentation.

Performance tests have been made with calibrated standards of ^{3}H and ^{14}C in the form of poly(methyl methacrylate) pieces with known electron emission defined as the number of electrons emitted per second per cm^{2}. Several thin-layer media have been used, mostly with directly applied spots of [^{3}H]- or [^{14}C]glucose solutions. Upon drying, spots of activities down to 5 nCi could easily be detected (spot areas were approximately 0.7 cm^{2}). A resolution of 1 mm could easily be achieved in the one-dimensional scans.

Some more results of a somewhat preliminary nature are reported. Some possible alternatives for the recording of measurements which use the channel electron multiplier detector are mentioned.

The use of scanning by electron multipliers as a method for radiochromatography has the advantages that the sample is neither damaged nor contaminated compared with conventional techniques which, for example, employ the admixture of wet (or dry) scintillators. It is felt that these advantages for certain applications more than compensate for the fact that the technique requires a vacuum of $5 \cdot 10^{-4}$ torr.

INTRODUCTION

In principle, a number of methods exist for radionuclide detection in chromatographic samples. In practice, however, the scintillation methods are still the most sensitive ones. In some cases there might nevertheless be a need for a system of detection which would leave the chromatogram undamaged and free from scintillator contaminations.

The purpose of this paper is to draw attention to a new type of electron detector which may have some advantages over gas-flow GM detectors for the radiochromatographic detection of tritium (^3H). The discussion is in general terms and rather short, as the system is still under investigation. While further development of the described system seems possible, there are some obvious disadvantages. A preliminary description of the system has been given earlier[1].

For the detection of radionuclides other than ^3H and perhaps ^{14}C, the sensitivity of ordinary gas-flow Geiger Müller (GM) detectors is sufficient. For ^3H detection, on the other hand, the lower energy of the electrons results in path lengths of only a few μm in the sample substances or a few mm in air (or in the counter gas). This is the explanation for the low counting efficiency for tritium (the proportion of counts per disintegration is only about 0.5–5%). In addition, a preliminary (unpublished) experiment showed a slightly increased ^3H-electron exposure of X-ray films when exposed in a moderate vacuum. These considerations suggest the use of an electron multiplier detector operated in a vacuum system.

It was also supposed that the reproducibility of such a system would be much less dependent upon the maintenance of a constant gap between the detector and the chromatograms. This was found to be correct. The vacuum requirement, however, excludes the investigation of substances which are too volatile.

In GM tubes, a signal amplification is achieved as the single incoming electron starts a cascade of electrons which, on their path towards the central collector electrode, held at a positive h.t., collide with gas atoms which in their turn, upon ionization, give off new electrons, thus steadily increasing the cascade. In this way single electrons reaching the detector from the spots of the radiochromatogram may be counted or, alternatively, a smoothed current may be recorded.

For the class of particle detectors termed electron multipliers, the required signal amplification is achieved by successive impingements of electrons on to secondary emitting surfaces or dynodes, held at increasingly higher potentials. Eventually, the electron cascade is collected at an anode. Again, pulse counting and current measurement are alternative possibilities.

For the described detectors to work adequately, the gas pressure must be held equal to or below $5 \cdot 10^{-4}$ torr. This is partly because unwanted effects occur if positively ionized gas molecules in appreciable numbers drift backwards in the multiplier tube. Corona discharges must also be avoided. Therefore, the chromatogram scanner had to be enclosed in a vacuum chamber together with the detector and the chromatogram. Several constructional details stem from this requirement.

METHOD

Fig. 1 shows a diagram of the β-detection equipment. The chromatogram is supported by a 20 × 20 cm table which can be moved in two perpendicular directions by means of pulse-controlled step motors. The scanner is contained in a stainless steel housing which may be evacuated to 10^{-6} torr by means of an ordinary oil diffusion pump. The signal from the detector is amplified and recorded. In two dimensions a modified x–y recorder might be used. The position reproducibility is of the order of 0.5 mm. The signal, in the form of amplified pulses, may be recorded either as a d.c. output signal from a pulse count-rate meter, or in the form of a histogram by use of a special scaler. In the latter case, the height of each column is proportional to the number of counts obtained in the corresponding position during the constant pre-set step time. Fig. 2 shows the vacuum scanner and the detector. Fig. 3 shows the whole experimental set-up. In the background (on the top of the rack) two units producing driving pulses for the step motors, which can be driven at various rates, can be seen. On top of these units is the h.t. power supply for the detector. One of the step motors with its reduction gear can be seen mounted on the left side of the chamber. The rotating shaft is coupled into the chamber through a vacuum seal. The electrical leads are taken through a vacuum seal situated on the top of the chamber along with the Pirani gauge head. The vacuum valves and the diffusion pump are hidden from sight on the right-hand side of the chamber. At the bottom of the photograph is the Philips rate meter which was used and in front of this is the x–y recorder. Beside this is situated the electronic equipment of the Pirani gauge. Behind this the rotary pump is just visible. The special scaler, depicted in Fig. 1, is not shown here.

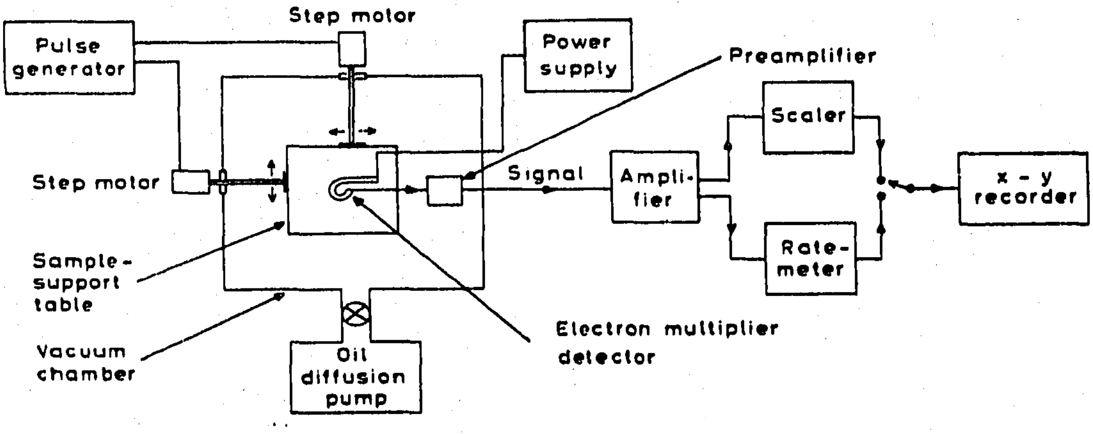

Fig. 1. Block diagram of the β-detection equipment. The step motor control unit and the electronic coupling of the recorder position to the detector position are not shown.

The vacuum system

The volume of the vacuum chamber is about 75 l. The walls are exclusively made from stainless steel and are of the order of 2 mm thick. A short tube of 10-cm bore connects the chamber to the oil diffusion pump via a baffle valve. A liquid nitrogen oil trap was planned but has been omitted without the introduction of complications. The pumps used are the "Speedivac" vapour diffusion pump model E 02 and "Speedivac" ES 150 high vacuum pump from Edwards High Vacuum Ltd., Sussex, Great Britain.

In the conventional manner, provisions were made for roughing the chamber by direct connection of the rotary pump during the initial phase of pumping. An Edwards Pirani gauge was used to measure the residual pressure in the chamber. One drawback was that the ionization gauge head could not be connected for measurement during the operation of the electron multiplier detector as the electrons produced by the Pirani head give an extremely large background count rate.

The mechanical units within the chamber were produced free from any large obstructed volumes and were made of materials that required a negligible degassing

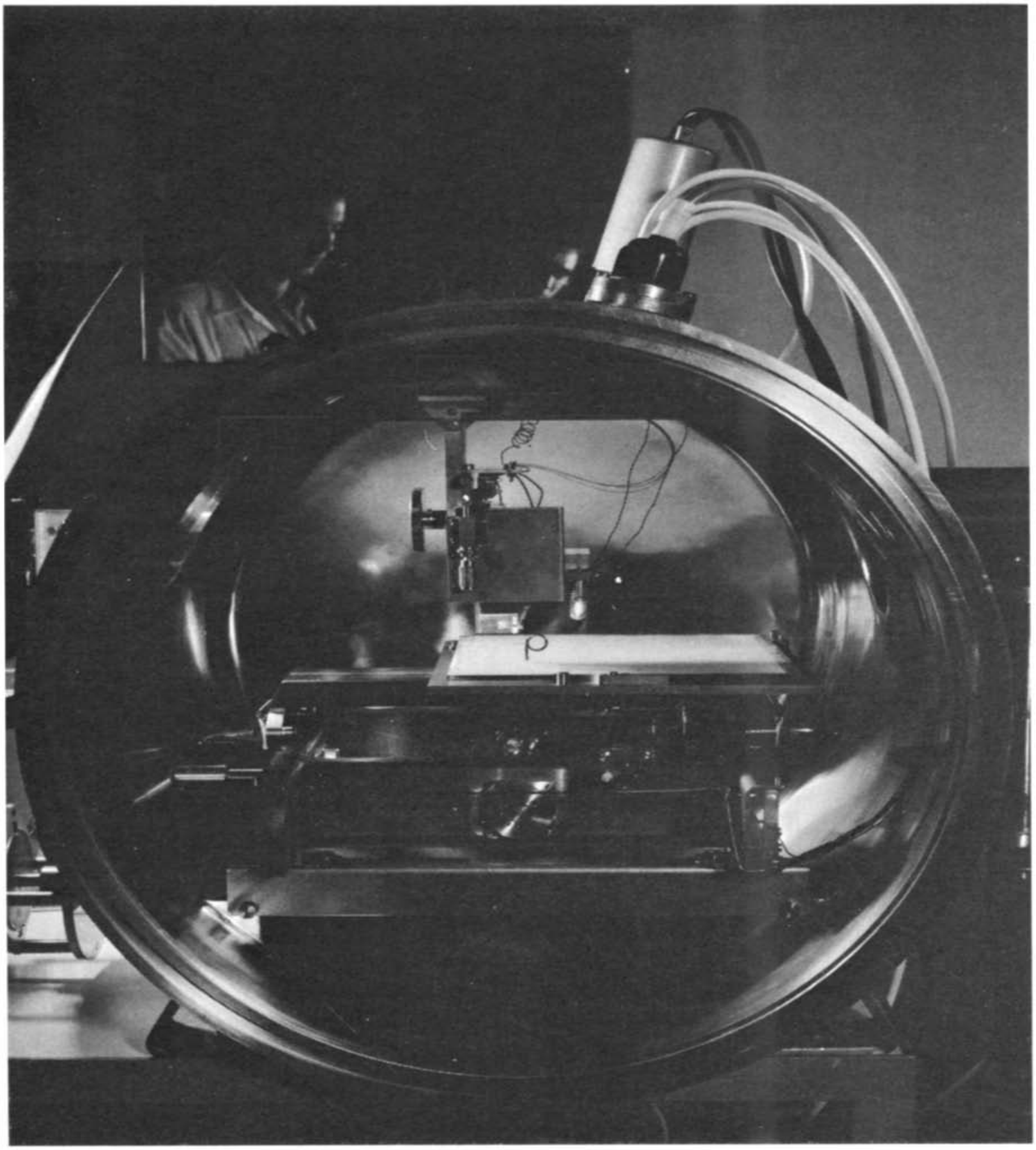

Fig. 2. The electron detector, closely coupled to its preamplifier, may be adjusted in height above the chromatogram which is seen fixed to the three-point support table of the vacuum x–y scanner.

period, after air admittance. During normal use, the required vacuum of $5 \cdot 10^{-4}$ torr can be reached in less than 15 min.

The scanning system

Two step motors are mounted outside the vacuum chamber with driving shafts coupled vacuum-tight through the chamber walls. An approximately 10:1 reduction gear is used. Inside the chamber, the moving table, 20 × 20 cm, can be drawn back

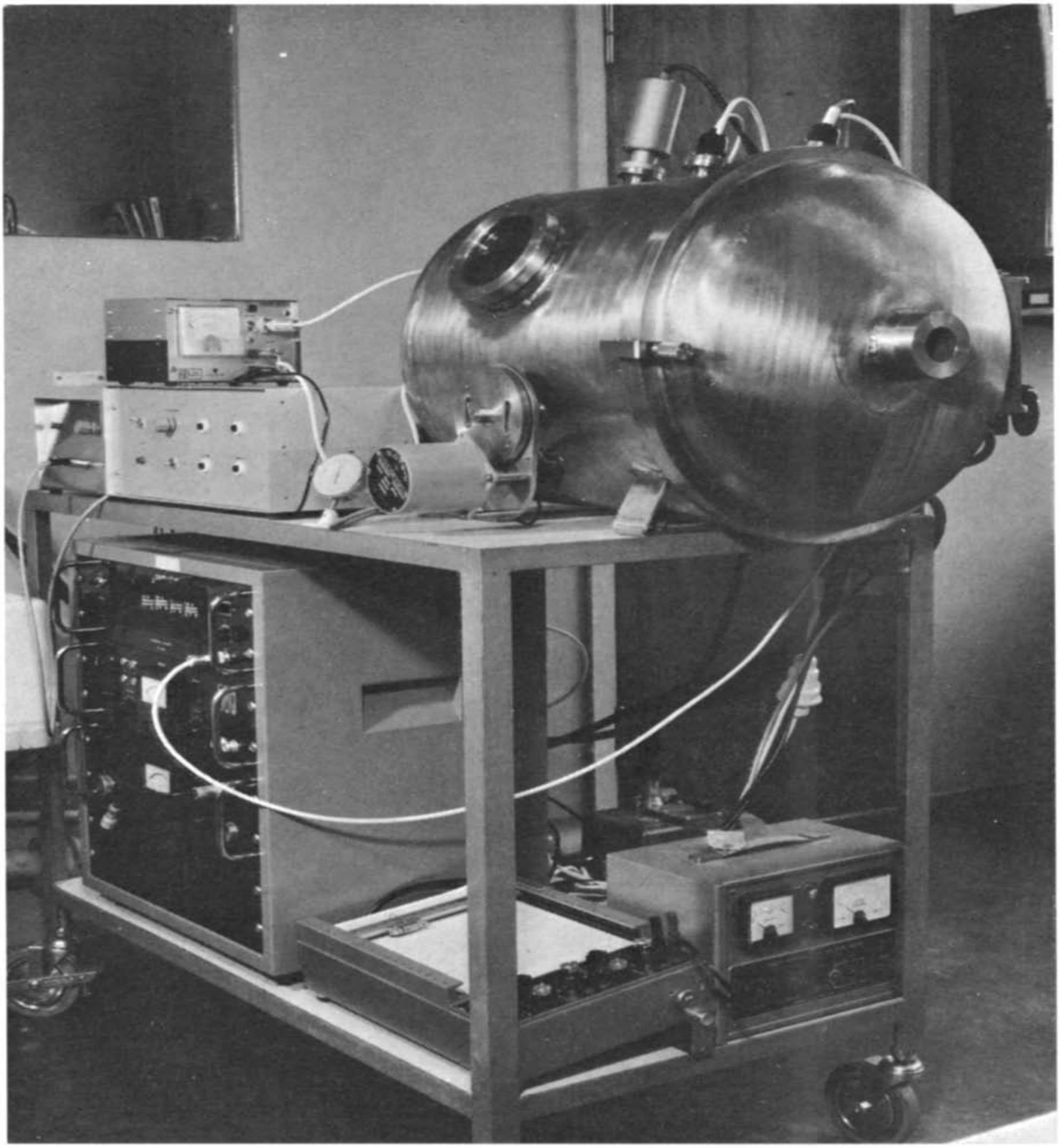

Fig. 3. The whole experimental set-up. For further description, see text.

and forth through 20 cm in two perpendicular directions by means of piano wire connected to rollers on the rotating shafts.

The motors are activated to move stepwise by means of electric pulses. Each step corresponds to *ca.* 0.3 mm table movement. Thus, a pulse generator is needed to produce the table movements. Some simple logic was added to perform automatic table operations. Using push-buttons, the table can be moved back and forth both in and perpendicular to the scan direction. Thus one can manually position the detector over a spot maximum and then start counting. In the automatic mode, microswitches actuate end stops in both the scan and the return directions. During the return of the table, a sideways movement of a selected number of steps is performed. In this way, an automatic scanning of a two-dimensional chromatogram may be made. This operation imposes some requirements on the data presentation method which are discussed in a later section. It is also possible to add the counts obtained during two or four steps in the forward direction. This enables one to make a fast scan to localise the spots, which may be counted later. The scan system thus performs fast movements in short steps and then the table rests in this position for a specific time which may be preselected as one of eight possible counting intervals. The available numbers of steps per min are 1, 2, 4, 6, 12, 30, 60 and 120.

Detector types

Many types of electron multiplier are now available. They are all based on the principle of electron cascading, either within a structured system of dynodes or along a single high-resistance dynode film supported by an insulating material. The factor of multiplication or gain is given very roughly as $G = n^m$, where n is the number of secondary electrons emitted from the dynode surface per incident (primary) electron, and m is the number of multiplying jumps along the surface of the dynode strip (or simply number of dynodes in the first type). G may be as high as 10^6 (or 10^8 in the first types). We have applied miniaturized detectors of the continuous dynode type.

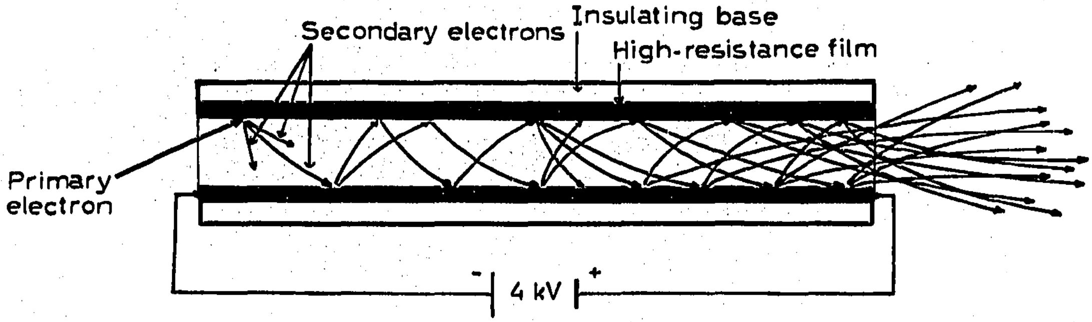

Fig. 4. Channel electron multiplier.

The channel electron multiplier is so called because the multiplying process takes place within a tube or channel. The first proposal for such a device was made by FARNSWORTH[2] in 1930. An electron cascade could, in principle, be produced by secondary emission from the internal wall of a tube of a resisting material, the ends of which are held at different potentials. An electron (or another particle) entering the low-potential end of the tube releases one or a few secondary electrons when striking the wall, as shown in Fig. 4. The secondary electrons are drawn towards a more positive potential and strike the opposite wall after some acceleration has taken place. New secondary electrons are released and the process continues along the full length of the tube. Contrary to conventional electron multipliers, which have an integral number of dynodes, the channel multipliers with their single continuous dynode strip have an indeterminate number of multiplication stages. In practice, the possibility of miniaturization is unique to the channel multipliers.

There is no evidence in the literature that FARNSWORTH succeeded in producing his suggested channel electron multiplier[3]. Thirty years elapsed while the technology of producing suitable resistive wall materials was developed. In 1960, OSCHEPKOV[4] and in 1962 GOODRICH AND WILEY[5] described working multiplier tubes and since about 1965 several experimental versions have been available from Mullard Ltd. The earliest available data were given by ADAMS AND MANLEY[3].

While these multipliers may be of the linear type shown in Fig. 4, they may take a variety of forms, provided that the length of the tube is kept equal to or larger than thirty times the tube diameter. It may be found for linear multipliers[3] that the gain is pressure-dependent owing to the feedback of positive ions which cause after-pulsing to occur. By avoiding the linear geometry, the pressure-dependence of the gain is removed[3].

The small dimensions of such detectors might be of importance when high spatial resolution is required. Likewise, the small dimensions of the counter are thought to be of importance for the total noise level. This has contributions from the radioactivity in wall materials, background radiation and thermally released electrons from the dynode coating. Mean noise count-rates less than 1 count per 10 min have been found in some detectors with no background screening other than the walls of the vacuum container made from 2-mm stainless steel.

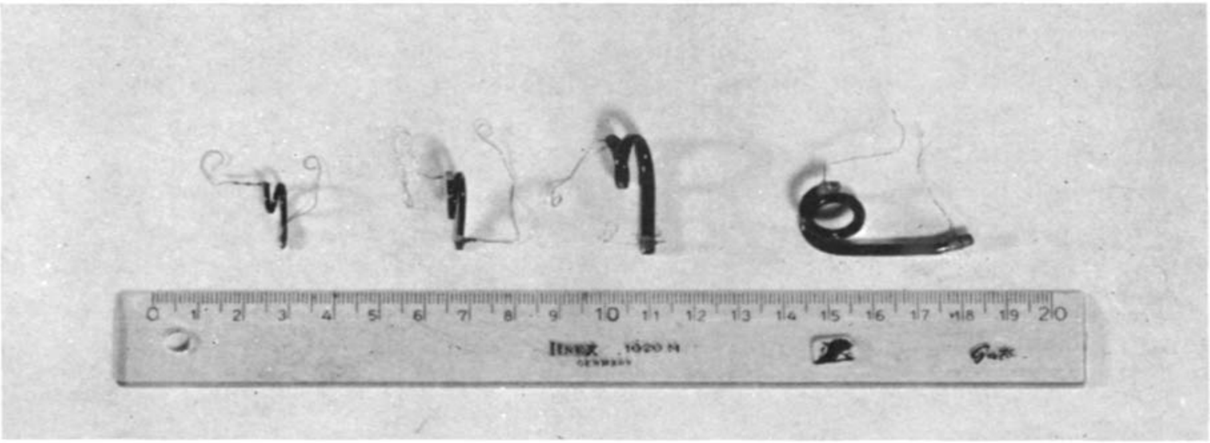

Fig. 5. Detectors. The right hand one is a side-slit detector with slit dimensions 1 × 5 mm. In addition, we used one with a 15-mm-long side-slit (not shown).

For one-dimensional scanning, we obtained from Mullard Ltd. two specially made detectors having a side slit instead of the standard end opening. One of these detectors is shown in Fig. 5 together with the standard versions. The end of the tube had to be tilted upwards so that the electrode connection did not intersect the plane of the radiochromatogram. The other side slit counter had a planar geometry and a 1 × 15 mm slit.

For two-dimensional scans, detectors with an end opening are the most useful. These have a straight cathode end with a circular opening like that shown in Fig. 4 and a curved anode end as may be seen in Fig. 5. We have used detectors with opening diameters 2.0, 1.2 and 0.7 mm. The smallest apertures might be best suited for high resolution scans, but at the same time the electron collection area is diminished, so that the resulting count rates are also low.

We thought it very important that the detector should scan very close to the surface of the chromatogram, so as to cover the greatest possible solid angle of electron emission from the spots. However, as indicated later, this requirement is not as strong as one might think.

Electronics

Charge pulses containing about 10^6 electrons are not very difficult to detect. However, a preamplifier and pulse shaper was coupled close to the detector. A positive h.t. of about 4 kV is applied to the anode end of the multiplier. The negative pulses are amplified and either counted in a special scaler, which will be mentioned below, or fed to a rate meter from which the corresponding d.c. signal can be recorded. However, the stepped motion of the scanner makes the former version the most suitable.

Data presentation

To obtain the most informative presentation of radiochromatographic data, the various possible methods should preferably be tested in practice. In this respect, however, our investigation must as yet be considered somewhat preliminary. The one-dimensional display systems are naturally very much simpler than the two-dimensional ones and in the first instance we shall concern ourselves with approaches of the former type.

In one dimension, the radiochromatographic strips may be scanned by rate meter recording of the variation of the count rate along the activity profile. This mode of operation is not very suitable because, as mentioned earlier, the scan must be carried out stepwise. For each 0.3 mm along the track, one will see a pen movement around some mean value of count rate with deflections being large for small time constants (RC)* and small for large time constants. Between these vertical "bars" there will be 0.3-mm-long horizontal "connections".

A more useful type of display is achieved in the form of activity histograms if one uses a special scaler connected to the detector. Our scaler counts for the preselected counting period and then at the end actuates the plotting of an x–y recorder whose y-position is determined by a d.c. voltage proportional to the count number of the scaler, then the scaler is zeroed, the radiochromatogram table moved one step to the next position, and the detector starts counting again. The counting capacity of the scaler can be selected as either 10^2, 10^3 or 10^4 counts. The same pulse generator controls the step motor for advancement of the chromatogram and the plotting of the count number. The procedure is repeated until the end stop of the chromatogram support table is reached. The pen movement along the x-axis is controlled by a ten-turn potentiometer connected to the drive shaft from the step motor of the scanner. Normally it is most suitable to obtain the activity profile with a 1:1 x-axis scale. The reproducibility of the x-position of the recorder is about 0.5 mm.

While the scaler histograms obtained in the counting mode are not distorted at fast scanning speeds, this effect may show up when the rate meter mode is applied. Here the integration time is determined by the time constant which must be suitably chosen. If the time constant is too large for the scanning speed applied, the spot profiles may be distorted. This is illustrated in Fig. 6. Part (a) shows a scaler histo-

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* R = resistance; C = capacitance.

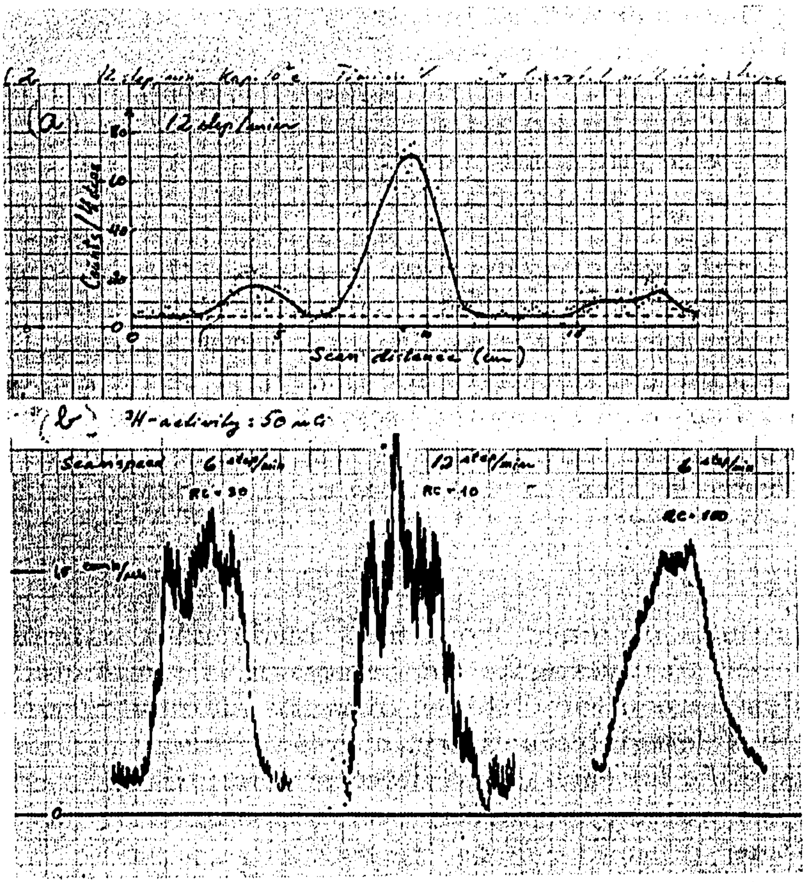


Fig. 6. Two ways of recording the scanned activity profiles. In part (a) a smooth curve has been drawn on the basis of a histogram plot where each dot indicates the number of counts reached in the preceding scan interval. Part (b) shows three examples of ordinary rate meter scanning of one and the same radiochromatogram spot. The scan speed and the time-constant of the rate meter have been varied. RC = 100 sec obviously is a too large time-constant as the profile has been tilted.

gram plot (by x–y recorder) and part (b) shows various rate meter-recorded spot profiles.

It would be an easy task to obtain a series of scaler print-out numbers instead of, or in addition to, the recording mentioned above.

The scanning of radiochromatograms where separation has been performed in two perpendicular directions is far more complicated. However, our scanner is suitable for this type of scanning. The whole chromatogram (20 × 20 cm) will, in the "auto" mode, be covered by parallel scans, the separation of which may be preselected. The difficulty is to find a useful way of presenting the activity readings.

One possibility would be to draw histograms, perhaps in different colours, along each track. However, this would probably result in a confusion of curves.

We have tried a different approach. Another paper[6] will describe the construction of a multisign plotter (fourteen different signs, seven in each of two colours) which may be mounted on the x–y recorder. In each position along the parallel tracks, count numbers may then be classified as belonging to one of fourteen consecutive ranges (with either a linear or a logaritmic scale) and the corresponding sign plotted. With a proper choice of track width, counter scale and sign types, the resulting activity chart might be intelligible. The scaler with an output in the form of fourteen different activation signals for the plotter is not yet available. Its construction, however, poses no unsurmountable problems.

It is, of course, possible to construct an iso-activity chart manually from a series of parallel scans and to get approximate total spot activities (from standardization experiments) for a radiochromatogram. An example of an iso-activity chart obtained in this way is given below.

It is an obvious step to consider the use of a computer to assist in the drawing of charts and evaluation of spot activities. Count numbers could be recorded directly on magnetic or paper tape as they are made, and the resulting tape used as input to a computer. We already have the equipment necessary for the direct recording of measurements onto paper tape, but a suitable computer programme still has to be written.

PERFORMANCE TESTS

We shall now report some results obtained from tests made with various miniaturized detectors. It is of interest to investigate the behaviour of parameters such as counting efficiency, dependence upon the distance from the chromatogram, minimum spatial resolution, minimum detectable activity, etc.

These parameters are partly specific for each of the detectors but still of direct importance for the performance of the whole system. However, all parameters have not been investigated for all detectors.

Test materials

The measurements have been carried out partly on ^{3}H and ^{14}C standards, in the form of thin plates of poly(methyl methacrylate), the electron fluxes of which have been calculated by the producer (The Radiochemical Centre, Amersham, Great Britain). In addition several thin-layer media have been used with active glucose solution applied directly as circular spots. Such experiments, where the activities can be easily controlled, are the most suitable ones for testing of the performance of the scanner. In addition, a few measurements have been made on one- and two-dimensional radiochromatograms, of the paper or thin-layer type (either cellulose, silica gel or alumina).

Solutions of various concentrations of [^{3}H] and [^{14}C]glucose were made up from standard solutions, available from The Radiochemical Centre, Amersham. Useful spot activities were made for the diverse tests by pipetting small volumes (5 μl) of the various sugar solutions onto Whatman chromatographic papers or thin layers of adsorbent materials. Spot areas ranged from 0.3 to 1.1 cm^2.

Even though the spots tended to show drying rings when applied to dry samples of adsorbing material, a sufficient reproducibility in the resulting count rate was obtained. In some cases, when they are scanned by a slit aperture, the drying rings are reflected in the recorded spot activity profile.

The poly(methyl methacrylate) standards also were obtained from The Radiochemical Centre, Amersham, and their strengths at the time of delivery were, for the tritium standard, TRP 3(10 mCi/g), 1.7×10^4 electrons/cm$^2\cdot$sec, and for the

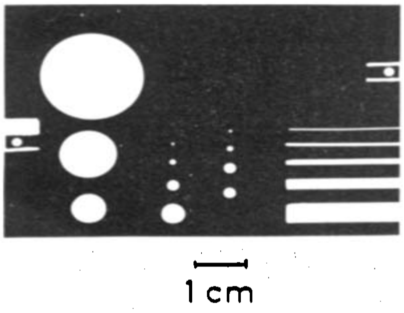


Fig. 7. Stainless steel mask of thickness 0.2 mm, intended for calibration measurements.

radiocarbon standard, CFP22 (528 μCi/g), 2.2×10^4 electrons/cm^2·sec. No data are available as yet for the electron energy distribution in the two cases.

In combination with the standards, a specially made mask of 0.2-mm stainless steel, containing series of holes and slits of systematically varying dimensions, was used (see Fig. 7).

Angular field of view

For a detector suspended over a chromatogram with its aperture pointing downwards, the angular field of view must be regarded as decisive both for the resulting count rate over an extended source and for the obtainable spatial resolution for a detailed activity distribution.

If it is assumed that the surface of chromatographic spots emits electrons with no preferential direction, it becomes useful to know how the probability of impinging electrons being counted, varies with the angle between their tracks and the normal to the aperture of the counter.

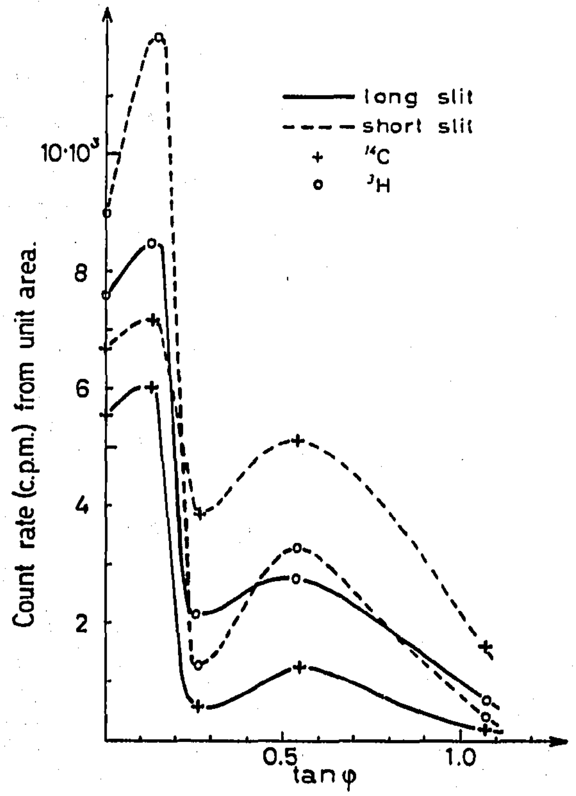
In order to investigate this point, a series of measurements were made using the previously mentioned thin metal mask in combination with the ^{3}H- and ^{14}C-standards. The mask was simply placed on top of the standards so that electrons were emitted through all the slits and holes. The slits were positioned one at a time vertically below, and parallel to, the aperture of the side slit counters and the count rates were noted. True count-rate differences divided by the corresponding differential slit areas yield the approximate angular dependence of the count rate for a unit area of isotropic electron emission. This dependence will not be corrected for the increase with the angle of the distance between the source element and the counter. This corresponds closely, however, to the normal application of the detector.

For the end-opening counters, having circular apertures, the holes in the frame were used in an analogous manner.

Two side-slit counters and two end-opening counters were tested. In both cases, one had a large and the other a small aperture.

In Fig. 8 are shown the curves obtained for the side-slit counters, with ^{3}H- and ^{14}C-electrons, respectively. The corresponding results for the two end-opening detectors are shown in Fig. 9. In both figures, the count rates are plotted against tanφ where φ is the angle between the source position and the normal to the counter aperture.

However, the curves shown are obtained with the poly(methyl methacrylate) standards, which are assumed to have an isotropic electron emission. It cannot be



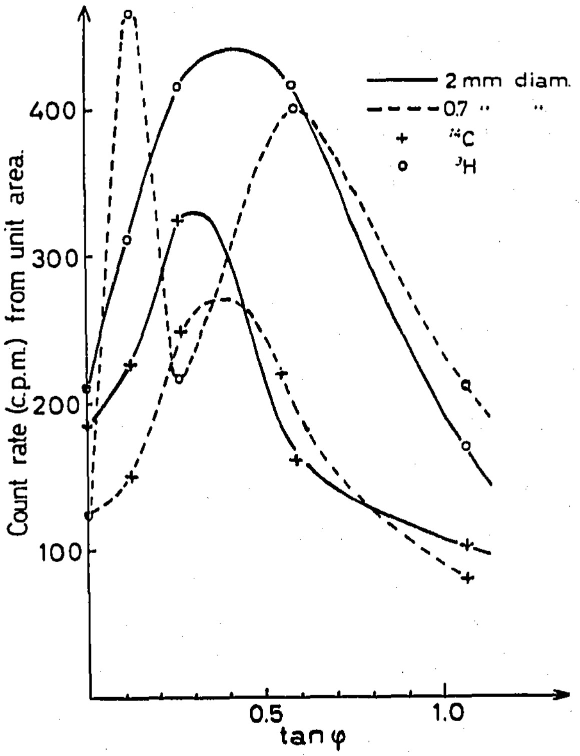


Fig. 8. Count-rate response of the side-slit detectors *vs.* the tangent of the angle between the detector normal and the direction to a line source element parallel to the slit. No corrections are made for the varying distance between the detector and the isotropic line source.

Fig. 9. Count-rate response of the end-opening detectors *vs.* the tangent of the angle between the detector normal and the direction to a circular zone element. No corrections are made for the varying distance between the detector aperture and the isotropic source element.

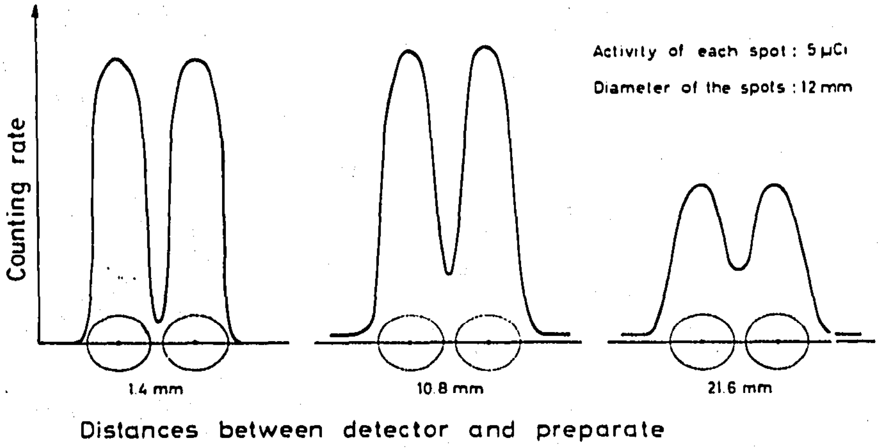


Fig. 10. The pen trace corresponding to a pair of ^{3}H-spots on Eastman Chromagram sheet (silica gel) for three different detector distances (1.4, 10.8 and 21.6 mm). On the same scale are shown the spots with their centres and drying rings. The distance between the drying rings is about 2 mm, their diametres about 12 mm and their activities 5 µCi (tritium-glucose).

taken for granted that the emission is such in the ordinary case where the activities are adsorbed on to paper or thin layers of cellulose, alumina or silica. In fact, our results seem to indicate that the electron emission is dominated by a component perpendicular to the surface of the alumina and silica preparations. This is discussed below.

Spatial resolution

As one might expect, the resolution of closely spaced spots decreases when the distance from the detector is increased. However, this decrease in resolution (and signal intensity) is much less than would be expected for spots with no angular variation in electron flux.

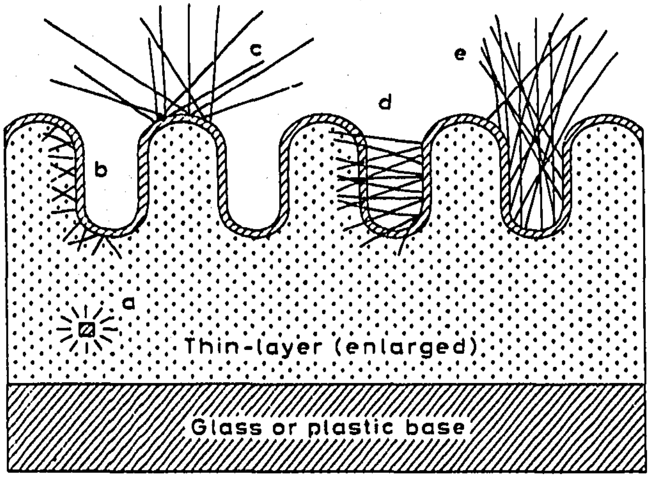


Fig. 11. Classification according to effectiveness of β-radiation from a spot of activity adsorbed on the surface of a thin-layer medium. For further description, see text.

The spatial resolutions obtained with three different counter-to-specimen distances for the same pair of ^{3}H-spots are shown in Fig. 10 for the large side-slit counter. It is assumed that the high resolution is due mainly to an angular variation in the electron flux, which is what one might expect for low-energy electrons emitted by an active substance covering a highly porous surface of a material with high stopping power.

We think, in fact, that the microgeometry of the electron radiation from activity spots in silica and alumina thin layers is better described in the form shown in Fig. 11. Possibly, the various spot materials tend to concentrate in the surface of the thin layers while the chromatograms are drying[7]. In any case, activity situated in the bulk of the layers (a) will in practice have a low efficiency, at least for tritium, owing to the small β-range associated with this nuclide. The β-radiation from the activity-covered grains and ducts in the upper surface may be classified as belonging to one of four categories: radiation inwards in a grain (b) will be ineffective. Radiation will be emitted from the top of grains (c) in all directions. However, a relatively small amount of active material is contained in such (c) positions. The same activity which is responsible for the radiation of type (b) will give radiation also of type (d), which will be absorbed in the neighbouring grains. Finally, active material on the walls and bottoms of the pores between the grains will emit electrons in almost vertical directions (e) which will not be absorbed in the layer but will instead be radiated from the spots of activity. We suppose this to be the reason for a prevailing vertical β-emission, and suggest this as an explanation for the results described in Fig. 10. To some extent, the narrowness of the peaks in Fig. 10 might reflect the sharp angular dependence of the counter as shown in Fig. 8. In the case of radiocarbon, the effect should be much less pronounced. This has not been investigated. One additional reason for the observed decrease in resolution when the detector distance is increased is that one component of the noise signal is found to be proportional to this distance. This component might be due to more or less isotropic background radiation inside the vacuum chamber. A theoretical discussion of the obtainable resolution or "fidelity" of radiochromatogram scans has been presented earlier by JOHNSEN[8]. Without going into such detail, we can state that a practically obtainable resolution with our scanner is of the order of 1 mm. This is judged from measurements made with the standards and the metal mask and also from the results shown in Fig. 10, both for the side-slit counter.

Linearity of detection

For practical applications, it is important to establish the detector response to various amounts of radioactive material contained in the chromatogram spots. A linear dependence of the recorded signal upon the activity is preferred. A series of four different ^3H activities, ranging from 5 to 5000 nCi, were made and their activity

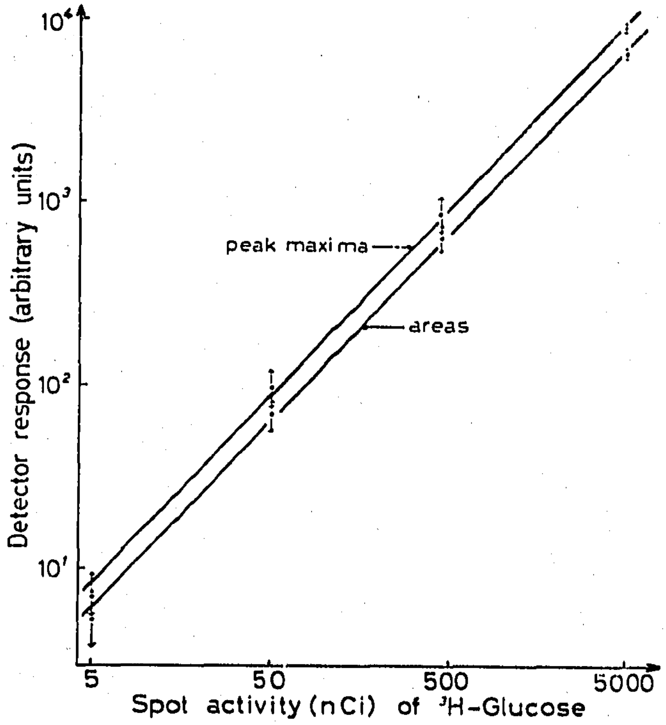


Fig. 12. Count response *versus* ^3H activity in nCi (log–log diagram). The responses are corrected for background counts. The upper curve represents count numbers in four minutes over the spot maxima. The lower curve represents areas under the spot activity profiles. (Eastman Chromagram sheet, silica gel).

profiles recorded, twice each. The results are given in Fig. 12. Two types of reading were made. In both cases, the maximum, mean and minimum values which could be read from the recordings are indicated. The count rates over each peak give the upper curve. The lower curve is drawn between corresponding values obtained as the areas under each peak. These (relative) values result from weighing the areas of recording paper after scissoring. In both cases the background signal was subtracted. Two straight lines could be drawn which always lay between the maximum and minimum values. These methods of controlling the linear response of a detector have been discussed earlier[7], where it was concluded that the comparison between areas is the preferred method, especially when evaluating spots of varying compounds and after different travel lengths in an actual chromatogram, which may cause the spot diameter to vary.

TABLE I

APPARENT COUNTING EFFICIENCIES OF FOUR THIN LAYERS WITH 5 μCi [^{3}H]GLUCOSE

The spot profiles are integrated to yield the given relative values. Correction for varying spot size has been attempted (see text).

Adsorbent type	*Relative apparent counting efficiency*	*Diameter of spot (mm)*	*Relative areas*	*Corrected relative apparent counting efficiency*
7736H, silica gel (Merck)	1	6	1	1
Whatman paper, SG81, AH81	2	9	2.3	0.9
Eastman Chromagram, K301-R2	3	12	4	0.8
7731G, silica gel (with gypsum) (Merck)	14	9	2.3	6.2

The apparent counting efficiency for a particular counter detecting tritium electrons was found to depend upon the type of adsorbent material or chromatographic paper used. This might be due to differences in activity deposition within the various media.

In Table I are shown the apparent counting efficiencies for four different adsorbent layers with a counter distance of 1.4 mm. The drying rings, being of slightly different diameters, indicate that the spot areas are different and thus that a somewhat varying amount of active material (glucose) may be deposited in the bulk of the layers, which may be of somewhat different thicknesses. The areas under the plotter curves for each of the spots have been integrated to yield the values given in Table I. It can be seen that the silica gel with gypsum from Merck gives a much higher apparent counting efficiency than the other media, and thus seems superior for low-activity measurements. In the last column are given apparent counting efficiencies corrected for varying layer thicknesses. It is assumed that a thick layer will give a small spot and little material will be absorbed at the layer surface. The corrected values have resulted from division by the respective spot areas. If surface concentration occurs during drying[7], the correction is too great and the true values will lie between the corrected and the uncorrected ones.

Background and noise

Even in the absence of radioactive material near the counter, there is still a background count rate. This is caused by the radioactivity in the counter wall materials themselves, the background radiation in the vacuum chamber, and the thermal release of electrons from the dynode strip coating. However, mean values of total noise count rates have been found as low as 1 count in 10 min with no background screening other than the walls of the vacuum container made from 2-mm stainless steel. This is an extremely low value compared with GM counters. It is certainly important to select materials free from radioactivity for the manufacture of the detector. Secondly, its very small volume compared with the sensitive volume of a GM counter is important for minimizing the influence of background radiation. More precisely, our detector has a sensitive surface layer (that of the inside of the mouthpiece) rather than a sensitive volume as in the case of the GM tube.

Total count numbers N_t and total count rates $\dot{N}_t$ must still be corrected by subtracting the corresponding background values N_b and $\dot{N}_b$ to yield the source-count and -rate, N_s and $\dot{N}_s$, respectively. However, due to the fact that both the source signal and the background are noisy in the sense that their values fluctuate due to their statistical origin, mean count rate values over some practical time interval must be established.

Assuming Poissonian statistics, the standard deviation, which may be alotted to an established mean count value as uncertainty limits, is given by:

$$\sigma_{N_s} = \sqrt{N_t + N_b} = \sqrt{N_s + 2N_b}$$

assuming no error in timing, and for count rates:

$$\sigma_{\dot{N}_s} = \sqrt{\dot{N}_t + \dot{N}_b} = \sqrt{\dot{N}_s + 2\dot{N}_b}$$

It is assumed here that the source signal can never be obtained free of its background, but the background with its standard deviation can be established by a separate measurement in the absence of the sample (source). The background count rate of the various detectors employed varied considerably. While it has been observed as low as 1 count in 10 min, it may for other detectors be sixty times greater.

The background count rate was found to consist of two components, one originating either from electrons released by field emission or due to radioactivity in the wall materials of the counter itself, or both, and the other possibly being due to thermal electrons traversing the vacuum chamber in all directions. The count level of the latter component was found to increase proportionally with the distance between the counter and the surface underneath.

During our experiments we have damaged one or two detectors by applying a high voltage in the corona range of pressure. This permanently increases the noise pulse rate.

We have also contaminated one or two detectors with ^{3}H-containing particles from the thin layers. The detectors could be washed with alcohol without being damaged, but we did not succeed in returning to the originally low background count rate. As demonstrated in the next section, a low background count rate is very important to the achievement of a low limit of detection. Owing to the fluctuating signal and background, faint activity spots become difficult both to locate and to measure. The manner in which the above standard deviations are reflected in percentage errors in estimated peak activities has been shown earlier[8]. We shall look somewhat closer into this in connection with the performance of our detector.

Limit of detection

Provided that the activity distribution can be represented by a Gaussian distribution and that the slit width (S) of the detector is small, so as not to increase in the recording the full width at half-maximum (H) of the spot profile (in the scan direction), one can express[7] the minimum detectable activity ($A_{\min}$) as

$$A_{\min} = 1.065\,\dot{N}_b \cdot H/S \cdot \eta.$$

Here η is the overall counting efficiency (c.p.m./d.p.m.) for a detector with no slit, so that all electrons travelling upwards are collected by the counter. We shall refer to this formula without going further into the theory behind it in order to show how important a low background count rate and a high counting efficiency are when one wishes to have a low level limit of detection.

For a GM windowless gas-flow counter, BLEECKEN *et al.*[7] found η equal to 42% (c.p.m./d.p.m.) for a spot of [^{3}H]cholesterol applied to a polished plate, while the value on a Silica Gel G (Merck) thin layer was as low as 1.6% owing to sample absorption. The corresponding value for [^{14}C]cholesterol was 47.4%.

For our particular detectors, it is difficult to establish η as the aperture is fixed in each case. However, the count rates may be compared for the various detectors over the poly(methyl methacrylate) standards. While it became important for BLEECKEN *et al.*[7] as well as for SCHULZE AND WENZEL[9] to decrease the distance between the chromatogram and the GM detector as much as possible, we have seen that this is no strong requirement in our case. We have, on the other hand, observed an angular dependence in the counting efficiency.

For our various detectors, relative values of count rates, corrected for background over the ^{3}H- and the ^{14}C-standard sheets, are given in Table II. The values are normalized by setting both efficiencies arbitrarily to unity for the large side-slit counter.

It can be seen from Table II that the various detectors have different counting efficiencies for electrons entering the apertures. It is difficult to establish a true

TABLE II

RELATIVE COUNT RATES OF THE VARIOUS DETECTORS

Detector type	*Aperture (mm)*	*^{3}H-electrons (TPR3 standard)*	*^{14}C-electrons (CFP22 standard)*
Side-slit counter, large	1 × 15	1	1
Side-slit counter, small	1 × 5	0.34	2.4
End-opening counter, large	Diam. 2.0	0.02	0.08
End-opening counter, small	Diam. 0.7	0.02	0.05

counting efficiency, η, by use of activities in thin-layer media because one does not know the proportion of the electrons from the disintegrations that actually reach the detector. By use of our standards with calibrated fluxes, the sample absorption is no longer a problem but difficulties still remain because of the angular dependence of detector response. However, for geometries resembling those of common scanning, we seem to get an approximate value for η of (40 ± 20)% for tritium (for the large side-slit counter). It is known that η decreases as the electron energy increases for this type of detector. According to FRANK, it is 60% for 450 eV electrons (*cf.* ref. 10).

The η value of 42% (c.p.m./d.p.m.) found by BLEECKEN *et al.*[7] employing a GM counter is substantially higher as they count 84% of the electrons entering the upper 2π solid angle covered by their detector (with no slit aperture).

However, the low level limit of detection is further proportional to the background count rate. Our types of counter have a very low background when they are not damaged or contaminated. Because of this, they may still compare favourably with the GM gas-flow windowless tubes. In our case, a tritium activity of 5nCi could be detected with a contaminated detector. This is the same limit as found by BLEECKEN *et al.*[7] for their GM counter. However, with an undamaged detector with a noise count rate as low as about 1 count in 1 minute, the limit would be even smaller in our case.

For the choice between counters, a criterion put forward by LOEVINGER AND BERMAN[11] may be applied: If E is the sensitivity or counting efficiency (c.p.m./d.p.m.) of the counter, the counter with the largest E will always be the best provided that it also has the largest $E^2/\dot{N}_b$. If that with the largest E has the smallest $E^2/\dot{N}_b$, it will be advantageous for (sufficiently) high spot activities and disadvantageous for (sufficiently) weak activities. This criterion, developed for the counting of radioactive samples, is also thought to be valid for detectors used in radiochromatogram scanning.

Finally, when double-lable experiments are performed, *e.g.* with ^{3}H and ^{14}C, one may scan the chromatogram twice, firstly recording both radionuclide activities and secondly detecting only the ^{14}C activities. This is achieved by means of a thin sheet of plastic covering the chromatogram. This effectively shields out the ^{3}H electrons owing to their low range in solid material, while the attenuation of the ^{14}C electrons will be negligible. The two resulting activity profiles or activity charts may afterwards be compared and the ^{3}H- and ^{14}C-activity spots located. This technique has been described by WENZEL and co-workers[12,13].

Two-dimensional scanning

As mentioned in the introduction, we have not yet combined two-dimensional scanning with a useful readout or printing system. Some methods which might be adopted in the present system were mentioned. A manually produced two-dimensional activity chart is presented here as an example of what may be obtained with the present scanner–detector assembly.

The chromatogram shown is made from an ethanolic extract of *Papaver somniferum*. The solvents water-saturated phenol–water (100:39, w/w) and butanol–propionic acid–water (8:18:12) have been applied successively on Whatman paper No. 1 in the y- and the x-(scan-) directions, respectively. Out of an area of 57 × 45 cm, we have selected a smaller one, 19 × 16 cm, which could be placed on the chromatogram support table and scanned. The labelling is with $^{14}CO_2$ as described elsewhere[14].

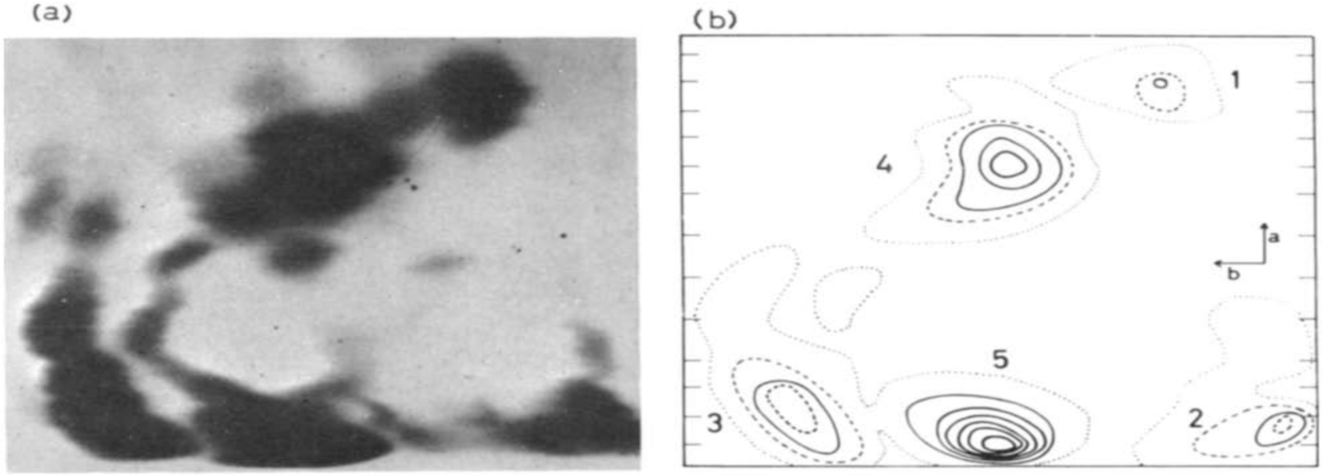


Fig. 13. Part (a) shows an autoradiogram of a two-dimensional radiochromatogram of an ethanolic extract of *Papaver somniferum*. The chromatography was performed firstly in the a- and secondly in the b-direction. Part (b) of the figure shows an iso-activity chart constructed on the basis of thirteen parallel scans, each of which has been indicated in the margin. Iso-activity contours have been drawn at intervals of 30 c.p.m. (———) and 15 c.p.m. (— — — —). An additional contour (·····) is drawn around each spot where the activity significantly increases above the background count rate. The various spots are: 1, aspartic acid (0.028); 2, meconic acid (0.031); 3, malic acid (0.052); 4, glutamic acid (0.068); and 5 citric acid (0.11). For each spot is given the integrated activity in percent of the total activity in the applied spot.

We have made 13 parallel scans of full length with a distance of approximately 1 cm between each. From the resulting activity profiles, one of which is shown in Fig. 6(a), the activity chart shown in Fig. 13(b) was constructed. A reproduction of the autoradiogram (Kodak Medical X-ray film, blue sensitive) is shown in Fig. 13(a) for comparison. The necessary exposure time for this was about 10 days. The total scan time for the 13 scans was 12 h. Isoactivity contours are drawn in the activity chart at intervals of 30 and 15 c.p.m. It is clear from this demonstration that useful two-dimensional scans may be produced automatically by the present equipment. Probably the computer-aided printout version is preferred.

CONCLUSIONS

The radiochromatographic detection of tritium-labelled compounds is still not successfully solved, but many methods have been proposed, see for example the review article by Pocchiari and Rossi[15]. All the detection methods discussed, both one- and two-dimensional, can be applied to ^{14}C and other radionuclides with higher β-energies, but for tritium most of these methods have a very low counting efficiency. This is also true of a development made by Hariharan *et al.*[16], which is effective in locating ^{14}C compounds in two dimensions. This development is based mainly on two older works[17,18]. In one[17], a GM counter which is scanned in parallel tracks over the two-dimensional chromatograms is used. In the other[18], an array of GM counters is placed along the track of one-dimensional chromatograms. By use of 30 counters spaced at 1 cm intervals, an activity profile 30 cm long and with a resolution of about 1 cm can be obtained 30 times faster than when one single scanned detector is used.

The method presented in this paper might have advantages for tritium detection firstly because the measurements are made *in vacuo*, thus eliminating the attenuation of the β-radiation between source and detector, and secondly (and we believe this to be the most important) because of the low background count rate of the electron multiplier detectors. However, the vacuum requirement can be somewhat cumbersome in practice.

A successful development of our principle of detection seems to give a higher resolution but a smaller sensitivity than another principle of detection which will be mentioned in conclusion. This new principle of detection has quite recently been applied in two commercially developed systems, one by Panax Equipment Ltd., Great Britain, called a spark chamber, and one by Baird Atomics Inc., U.S.A., called a β-camera. The first, relatively cheap, model provides a photograph of the spark pattern in a thin, flat, ionization chamber volume located closely above the chromatogram. The other, rather expensive unit provides various choises of photographic and electronic readouts. This version is based on the simultaneous use of 1622 individual windowless gas-flow GM counters. The β-camera has been briefly described by SNYDER[10].

A paper giving a more thorough discussion of available detection methods in radiochromatography is under preparation, and the emphasis there will be upon the localization and measurement of tritium activities.

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