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A SEMI-AUTOMATIC DEVICE FOR MEASURING RADIOACTIVITY ON TWO-DIMENSIONAL PAPER CHROMATOGRAMS

V. Moses and K. K. Lonberg-Holm

March 1962

(Lawrence Radiation Laboratory, University of California, Berkeley, Calif., U.S.A.) - A technique has been developed for the automatic measurement of radioactivity on areas of paper from paper chromatograms. The appropriate areas are cut out from the chromatogram and sandwiched between two continuous bands of thin plastic polyester film in an apparatus designed for this purpose.

In another apparatus the accumulated double band of polyester film bearing the chromatogram spots is automatically passed in a discontinuous manner between two closely-opposed detector tubes. Each spot remains stationary between the detector tubes for a pre-determined counting period. For each chromatogram spot the number of counts recorded, together with the elapsed time, is automatically printed onto paper tape. The double band of polyester film is then automatically advanced until the next spot lies between the detector tubes, and the cycle is repeated.
A Semi-Automatic Device for Measuring Radioactivity on Two-Dimensional Paper Chromatograms

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(Running title: Automatic counting of radiochromatogram spots.)

The automatic measurement of radioactivity in spots of labelled compounds on two-dimensional paper chromatograms is technically much more difficult than performing the same task with one-dimensional separations. In the latter instance resolution is required in one dimension only (i.e. along the direction of solvent travel) and with a suitable slit-width of the masking shield, speed of movement of the chromatogram past the detector and sensitivity of the detecting and recording equipment, a resolution can be achieved similar to that obtained with X-ray film radioautography, while simultaneously obtaining a continuous quantitative estimate of the radioactivity on the paper. The record may be obtained either as a continuous count-rate tracing with a pen recorder, or as a printed tape if the pulses received for pre-determined intervals of time are integrated and automatically printed. Such machines have been in use for many years and are available commercially.

Two-dimension chromatograms present an entirely different problem. Unless spots on a chromatogram are widely separated, resolution adequate to distinguish between them is extremely difficult to obtain by any type of scanning technique. The smaller the detector the higher the resolution, but the lower the number of events detected per unit time. To achieve sufficient resolution to distinguish between adjacent compounds on chromatograms of the type routinely obtained in this laboratory (Plate 1),
in which many spots lie within about 2 mm. of each other and frequently have irregular margins which may even dovetail together, the detector would need to be no larger than about 1 mm. in diameter. The number of emitted particles entering such a minute detector per unit time would be relatively small, demanding greatly prolonged counting times at each position in order to achieve any reasonable statistical accuracy; alternatively, such accuracy must be sacrificed. As one cannot predict the most suitable directions in which to scan the chromatogram unless the distribution of the spots is known (in which case scanning becomes superfluous), it is inevitable that no matter in which pair of dimensions at right angles to each other the scanning is performed, resolution will be incomplete unless spots are sufficiently widely separated and there is no dovetailing at the margins.

The difficulties of attempting simultaneously to scan and evaluate automatically a two-dimensional radiochromatogram are well illustrated by the apparatus designed by Chain et al. (e.g. Pocchiari & Rossi, 1961). They used a pair of opposed side-view Geiger tubes with a window size of 1 cm². Pocchiari & Rossi (1961) do not mention the distance between the two windows, but in a commercial model based essentially on this design (Bi-Dimensional Scanning, Model 460, Packard Instrument Company, Inc., La Grange, Illinois, U.S.A.), in which the window size may be set at 8.4 mm. x 10.2 mm. or 8.2 mm. x 10.2 mm., the distance between the two windows is 5.6 mm.; this in itself is quite sufficient to ensure that the resolution would be far poorer than 2 mm. We have demonstrated conclusively that a radiochromatogram similar to the one shown in Plate 1 cannot be scanned and evaluated quantitatively with the Bi-Dimensional Scanner; all the compounds numbered 1-8, for instance, were recorded as only two spots.
Most workers faced with this problem have located the exact positions, sizes and shapes of the spots on radiochromatograms by radioautography, and it continues to be our experience that this is the most satisfactory method for obtaining the location of labelled compounds with sufficient accuracy and resolution. Equipment has been designed to locate radioactive spots by making a facsimile of the chromatogram (Wingo, 1954; Aronoff, 1956; Perkins & Tyrrell, 1961). In the design by Aronoff (1956) and Perkins & Tyrrell (1961), a Geiger tube is moved across the chromatogram which is fastened to a drum, while simultaneously an electrically energized stylus is moved over electrosensitive paper wrapped around the opposite end of the drum. Each pulse received by the Geiger tube from a certain point on the chromatogram is recorded as a black dot on a corresponding position on the electrosensitive paper. In this way a facsimile of the radiochromatogram resembling a radioautogram is produced on the electrosensitive paper, though with a somewhat reduced degree of resolution. The main advantage of such a technique is the reduction in time required to produce a facsimile of the radiochromatogram compared with the exposure required for an X-ray film. Perkins & Tyrrell (1961) illustrate one facsimile requiring 2 hr. for preparation compared with the corresponding radioautogram exposed for 192 hr. The facsimile scanner is thus mainly of value when the location of the radioactive compounds on a small number of chromatograms is urgently required, since 10, 50 or 100 radioautograms could have been exposed in the same 192 hr., yet scanning would have occupied 600, 3000 or 6000 hr., respectively, if only one machine were available. Such comparisons are admittedly only approximate, since a radioautogram might require any period from minutes to months for adequate exposure.
Having located the positions of the radioactive compounds on the chromatogram, two main types of technique are used to determine the radioactivity in each compound. In the first the compound may be eluted, and after suitable manipulation, assayed on a planchette, in a scintillation counter or by conversion to some volatile compound and counted in a gas counter; or it may be counted by one of these methods without previous elution from the paper. Each of these requires time, limits the number of samples which can be handled to a low figure, and frequently results in a loss of the sample for further manipulation, though many of the counting devices have high efficiencies and a count is obtained of correspondingly high accuracy.

In the second technique (reviewed by Moses & Edwards, 1960), a large diameter (2.25 in.) end-window Geiger-Müller Tube (Fuller, 1956), fitted with a thin replaceable "Mylar" polyester window (density about 1 mg./cm.²) and flushed with an appropriate counting gas, is placed on the chromatogram at each position known to be the location of a radioactive substance. The number of counts from each location is recorded for a given period, often 1 min. Many spots on the chromatogram are frequently too large to be counted with the detector tube in a single counting operation, and part of the spot must then be shielded in a suitable manner (index cards are used for $^{14}C$) while another part is counted. The position of the shield is then moved so that the area previously shielded may be counted and the area previously counted is shielded. Because of the sizes of many spots, it is frequently necessary to count them in several, rather than in two operations. Spots on the chromatogram lying close to the one being assayed must also be shielded lest they interfere with the one being counted. Finally, a spot will usually give a different count on the two sides of the paper, probably due to differences in the rates of evaporation from the two surfaces of the solvent used for
development of the chromatogram. It is therefore necessary, in all investigations requiring a reasonably quantitative treatment, for each spot to be counted on both sides of the paper.

A typical chromatogram obtained in an investigation of the pathways of intermediary metabolism may have as many as forty or fifty spots on it (Plate 1). Perhaps one half of these spots will be too large to be counted in one positioning of the Geiger tube, and will need to be counted in sections. Thus, on both sides of the paper, there may be a total of more than 120 different positions to be counted, each, say for 1 min., and in practice 30 sec. must be added to each counting period for aligning the shields and recording the results, making some 3 hr. in all for each chromatogram. It is difficult to keep more than one counter in operation for 1 min. counts as the operator rapidly becomes confused and overall efficiency and accuracy of placing the masking shields deteriorates considerably. Equally important is the general tedium of the whole operation, and although it may be theoretically possible to count three such chromatograms in one working day, this places an undue mental strain on the operator, again with a fall in efficiency and an increase of carelessness. In addition, a 1 min. counting period is insufficient to achieve a high degree of statistical accuracy, while increasing the counting period to, say, 5 or 10 min., would slow the whole operation down to a quite unacceptable degree if this were done manually.

Some alleviation of these difficulties can be obtained by cutting out the spots from the chromatogram and placing them one at a time, if necessary in sections, between two Geiger tubes mounted with their windows opposed, and connected in parallel to one scaling unit. This eliminates the need for careful masking of parts of the spot not being counted, and of adjacent spots, while at the same time enabling both sides of the paper to be counted simultaneously (Moses, unpublished). However, once the spots have been cut out it then becomes possible to count them sequentially in an entirely
automatic apparatus, the description of which forms the purpose of this communication.

PRINCIPLE

Two pieces of equipment are required: a "loader" and a "counter".

Loader: The principle of the loader is shown in Fig. 1; a photograph is shown in Plate 2. A strip of Mylar (E.I. DuPont de Nemours & Co. (Inc.), Wilmington, Delaware, U.S.A.) 5 in. wide and 0.00025 in. thick, is unwound from a spool (1) along a table (2). The Mylar passes over a window (3 in. diam.) in the table (3), illuminated from below by an electric light bulb, and then runs between two opposed rubber-covered rollers (4). At this point another similar strip of Mylar from a second spool (5) is also fed between the rollers, and comes to lie exactly on top of the first Mylar strip. The double band of Mylar passes over another roller (6) and is wound onto a large take-up spool (7). Two felt pads (8) remove wrinkles from the Mylar. A strip of kraft paper, also 5 in. wide, is simultaneously unwound from a spool (9), and wound onto the take-up spool (7) together with the now double Mylar strip. At a particular position in relation to the illuminated window (3) is mounted a tape dispensing machine (10), operated by a foot pedal (11), which dispenses small rectangles of opaque adhesive tape onto the bottom strip of Mylar before it is covered with the upper strip.

The chromatogram spot, cut out of the whole chromatogram paper, is placed on the stationary lower strip of Mylar over the illuminated window (3), with a small drop of fast-drying glue applied to the leading edge of the spot. The glue serves to attach the paper spot securely to the Mylar and also helps to keep the upper and lower layers of Mylar in a constant relation to one another. A piece of opaque tape is dispensed from the tape dispenser (10) and the Mylar strip is then advanced by means of the motor (12), controlled by a foot pedal (13), operating on the rim of the take-up spool. The motor (12) can be operated continuously or only when the foot pedal (13) is depressed.
As the Mylar is so thin, it has very little resistance to bending and folding. It is therefore important that the axles of all the spools and rollers are accurately aligned or the combined strip will not wind uniformly onto the take-up spool. The purpose of the kraft paper is to provide a continuously smooth surface on which to roll up the combined Mylar strip onto the take-up spool. As chromatogram paper (Whatman No. 4) is about 15 times as thick as the double layer of Mylar, and the chromatogram spots are confined to the centre of the strip, a bulge develops in the accumulated strip on the take-up spool unless the backing of kraft paper is provided.

Counter. In the counter (Fig. 2; Plates 3 and 4) the strip is unwound from the loaded take-up spool (1), which has been transferred from the loader, the kraft paper (2) is separated from the double layer of Mylar (3), and the latter, after alignment by passage over two rollers (4), is passed between two end-window gas-flow counter tubes (5). The Mylar is passed over two more rollers (6), brought into contact again with the kraft paper and the two taken up together as in the loader onto another take-up spool (7) driven by a motor (8). A photoelectric cell (9) and a light (10) are so mounted that they bear the same relation to the centre of the counter tubes that the opaque tape-dispensing machine (10, Fig. 1) bears to the illuminated window (5, Fig. 1) on the loader. Thus, when a piece of opaque tape blocks light coming into the photoelectric cell, and a chromatogram spot lies exactly between the two counter tubes, the drive motor (9) is automatically switched off and counting commences. The output from the counter tubes in parallel is fed into a scaling, timing and automatic print-out circuit. The chromatogram spots are counted according to a pre-determined programme, and the elapsed time and recorded counts automatically printed out onto tape.
CONSTRUCTIONAL DETAILS

(a) Mechanical.

Sectional and plan diagrams showing the details of the design and mounting of the counter tubes are shown in Figs. 3 and 4. The brass plates holding the counter tubes are mounted in the frame 0.063 in. apart. The tubes are flushed with a gas mixture containing 0.99% (v/v) isobutane in helium (The Matheson Co. Inc., East Rutherford, N.J., U.S.A.). A manometer is placed on the outlet side of each counter tube to measure the gas pressure within the tubes. The counting gas is exhausted to the atmosphere via tubes containing glycerol about 0.75 in. deep. These bubbler tubes prevent air diffusing into the counter tubes and also enable a measure of the gas flow rate to be made. The Mylar used for the windows is 0.00025 in. thick, and is sputtered on each side with metallic gold (Atomic Development & Machine Corp., College Point, N. Y., U. S. A.). The overall density of the metallized Mylar is 1.0 mg./cm.².

The large spools for winding up the Mylar tape containing the chromatogram spots together with the kraft paper have wooden cores 5.125 in. wide and 6 in. diam., and flanges of aluminium 2.14 in. diam. and 0.1275 in. thick. The axles of these spools are held in "Gilito" bearings which are mounted on the main framework of the counter assembly.

A knurled disc (14, Fig. 2) is mounted on the axle of the take-up spool and serves as a friction drive from a 9.6 rev./min. motor (7 lb. torque). The drive shaft of the motor is fitted with a 1 in. rubber tyre which rests on the knurled disc. The motor is mounted at the end of a long metal arm fixed to the frame with a pivot as shown in Plates 3 and 4.

(b) Electrical.

In the operation of the sample changer control, a command pulse is sent
from the scaler on completion of counting (preset time, preset count, or time/count) to the printer and to the control box on the counter assembly; the latter starts the drive motor and turns on the light for the photocell circuit. After being switched on, the motor drives for at least 2.2 sec., regardless of the light beam, to permit the opaque tape to clear the photocell. The motor runs until the light beam is interrupted by a piece of opaque tape; this stops the motor drive by removing the power and applying a D.C. dynamic brake, switches off the light, and starts the timer and scaler.

The output signals are taken independently from each counter tube, and a selector switch (11, Fig. 1) selects either or both tubes.


Loader:

The take-up spool of the loader is driven on its outer rim by a motor (42 rev./min., 7.1 lb. torque) fitted with a 1.25 in. tyre. The motor is mounted on a hinged plate and the drive wheel is pressed against the rim of the take-up spool by the weight of the motor.

The opaque tape is dispensed by a "Trig-a-Tape" Model T-1 (Dymo Industries, Inc., Berkeley, Calif., U.S.A.). This is mounted on an aluminium bracket and is operated by a rod connected to a foot pedal. This machine dispenses pieces of tape 0.5 in. x 0.75 in., the long direction of the
piece of tape being at right angles to the direction of travel of the Mylar tape.

The two layers of Mylar together with the chromatogram spots are pressed together by being passed between two rubber-covered rollers, each 1.875 in. dia. The ends of the two roller axles are mounted 3.75 in. apart in an "Gilite" bearing block. The positions of these bearing blocks are adjustable to permit alignment of the Mylar strips. The two supply spools of Mylar are similarly mounted on adjustable bearings.

OPERATION

Loading. At the start of the loading operation the supply spools of Mylar and kraft paper are placed in their respective positions, and the take-up spool mounted correctly so that it can be driven freely by the rim-drive motor. Kraft paper is unsound from the supply spool and attached with adhesive ("Scotch") tape to the core of the take-up spool. The latter is then driven for a sufficiently long time to ascertain that the kraft paper is running true and is not fouling on the flanges of the take-up spool. The two strips of Mylar are fed through the double rollers and attached one at a time by adhesive tape to the kraft paper already on the take-up spool. At this stage the drive motor is switched to continuous motion and allowed to run until it is certain that the Mylar strips are running true; i.e., that the upper layer lies exactly over the bottom layer, that both layers are taut, and that the double layer is not fouling on the flanges of the take-up spool. The motor is then switched to control by the foot switch.

The chromatogram spots are, of course, counted in the reverse order from that in which they are loaded. The spots are loaded onto the lower strip of Mylar in a programmed order, a record of this order being
kept by the operator. With the Mylar tape stationary, a drop of glue
(such as DuPont "Froo" cement) is applied to the leading edge of the
chromatogram spot and this is then placed on the strip of Mylar at any
position within the circumference of the illuminated window on the loading
table. The Trig-a-Tape machine is operated by the foot pedal, the tally
counter is operated to record the loading of the chromatogram spot, and then
by means of the foot switch the Mylar tape is advanced until the spot
just loaded has been moved well clear of the loading position (about 5 in.).
The cycle of loading operations is repeated until all the spots have been
loaded. The loading cycle takes 5 sec. when the take-up spool is empty and
about 1.5 sec. when it is full.

Spots too large to fit within the permitted 3 in. diam. circular
window may be cut into sections, each of which is counted separately. In
some cases (e.g., long, narrow spots), the sections may be wound next to
each other if there is sufficient room to do so. At appropriate intervals no
chromatogram spot is placed on the tape, or a piece of blank paper is
loaded, in order to provide a background count. The stability of the counter
assembly is such that it is not usually necessary to incorporate background
counts more frequently than every 1 or 2 hr., unless a considerable
variation in background activity is expected (v. infra). After loading
is complete several ft. of blank Mylar strip and kraft paper are wound
onto the take-up spool. Both Mylar strips are then cut, and a few feet
more of kraft paper are wound onto the spool. The blank Mylar and extra
kraft paper are used as loading strips for starting the counting operation.

With the size of take-up spool currently in use (24 in. overall diam.,
6 in. o.d. diam.), the total capacity is about 3000 ft. of combined tape
plus kraft paper, or about 30,000 spots at 5 in. intervals.
Counting: The take-up spool is removed from the leader, and placed in
the supply position of the counter assembly (1, Fig. 2). The leading strip
of kraft paper is unwound from the supply spool, passed under a roller
at the bottom of the main supporting frame (15, Fig. 2), under the lower
counter tube and a further roller (16, Fig. 2), and is attached with
adhesive tape to the take-up spool. The motor drive wheel is raised
clear of the knurled drive on the take-up spool (14, Fig. 2), and the
kraft paper is wound onto the take-up spool by hand until the beginning of
the Mylar tape appears on the supply spool. Both layers of Mylar are
next attached by adhesive tape to a leading strip of kraft paper 5 in.
wide and about 2 ft. long. This leading strip is threaded over the first
guide roller and under the second (4, Fig. 2), between the two plates
holding the counter tubes, and then under the first and over the second of
the second pair of guide rollers (6, Fig. 2). The leading strip is then
pulled towards the take-up spool until both layers of Mylar have been
drawn through the counter housing and up to the core of the take-up
spool. The leading strip is then cut off the Mylar, and with the supply
spool held stationary, the take-up spool is wound on by hand until the
kraft paper extending from the supply spool to the take-up spool is pulled
taut. Tension is applied to the bottom layer of Mylar and when this is
taut it is attached with adhesive tape to the kraft paper already on
the take-up spool. The upper layer of Mylar is separately pulled taut and
similarly attached to the take-up spool. It is important at this stage
that both layers of Mylar should be equally taut, or a wrinkle will
develop on the less taut of the two layers which will eventually cause
the double layer strip to deviate from its correct path. The motor drive
wheel is now placed back onto the knurled drive wheel of the take-up spool. The drive motor is switched on and allowed to run until the operator is satisfied that both the Kraft paper and Mylar tape are running true. If adjustment is needed this is done by means of the guide rollers (4 and 5, Fig. 2).

The scaler, timer and high voltage supply are switched on and allowed to warm up. The gas supply valve is opened and the counter tubes are flushed with the counting gas. After a suitable period for flushing, each counter tube is separately checked for satisfactory operation by repeated background measurements. When these are constant at a level of activity known by the operator to be typical for the particular apparatus, the input selector switch is turned to the "both tubes" position and the background counts for both tubes operating together is checked repeatedly. The gas pressure (about 4 in. of ethanol above atmospheric) and gas flow rate (about 1 bubble/sec./tube) are checked, the time and count selector switches on the timer and scaler set to the desired values, the printer switched on and the sample index number reset to 1. Finally the drive motor is switched on, activated manually, and the scaling circuit set to the count position. When the first counting position on the Mylar tape reaches the counter tubes, activation of the photoelectric cell switches off the drive motor, counting automatically commences and proceeds for the preset time or count, whichever is reached first. As each sample is counted the print-out prints the sample index number, the number of counts recorded, and the elapsed time. On completion of each count, and printing of the count data, the Mylar tape is automatically advanced to the next counting position and the cycle is repeated until all the
spots have been counted. The Mylar tape and kraft paper are cut through and removed from the spools. The chromatogram spots may, of course, be recovered from the Mylar tape.

In the longest loading operation to date, 1250 counting positions were loaded onto the tape. The counting of this tape took 8 days to complete and required no attention on the part of the operator except to renew a cylinder of counting gas.

PERFORMANCE

Characteristics of Counter Tubes

Due to the large size of the counter tubes we have carefully investigated their characteristics.

Gas flow and pressure. The nominal distance separating the windows of the two opposed counter tubes is 0.063 in. The pressure of the counting gas inside the tubes is maintained at about 10 cm. of ethanol above atmospheric, this being controlled from two manometers on the outlet side of each tube (Plate 3). This pressure is sufficient to bow out the flexible Mylar windows of the counter tubes towards each other until between them they compress the Mylar tape bearing the chromatogram spots, thereby ensuring that the sensitive volumes of the counter tubes are brought into the closest possible contact with the Mylar tape, and simultaneously smoothing out any wrinkles which arise in the tape as it is wound from one spool to the other. By temporarily fitting the counter tubes with transparent plastic tops, it was possible to confirm that these wrinkles were indeed removed from the Mylar tape while it lay between the counter tubes. A gas flow rate of 1 bubble/sec./tube is sufficient to maintain the proper gas composition inside the counter tubes.
Lifetime of windows. The metallized Mylar windows of the counter tubes have withstood the movement past them of at least 2500 ft. of Mylar tape with no damage save for slight abrasion marks parallel to the direction of movement of the tape. As a general precaution against grit and dust falling on the exposed part of the tapes, with possible damage resulting to the windows, the whole counter assembly is kept permanently under a plastic dust cover stretched over a wooden frame, except when spools are being loaded onto or unloaded from the apparatus.

Length of anode probe wire. The central anode wire of each counter tube terminates in a glass bead. As the anode assembly is mounted in the roof of the counter tube on a screw-threaded insert, the distance from the glass bead to the window may readily be adjusted. The most satisfactory value for this distance has been found to be 0.25 in. from the bottom of the glass bead to the window.

Plateaux of the counter tubes. Using an input sensitivity of the scaler of 20 nV, the plateau characteristic of each tube, and of both tubes together, was measured with a sample of $^{14}C$ glucose on chromatogram paper placed centrally between the two tubes. The high voltage applied to the anode was advanced in 25 V intervals and the sample was counted for 1 min. at each voltage between the threshold and discharge potentials (Fig. 5). While the plateau was somewhat longer for the bottom tube (325 V) than for the top tube (225 V), the tubes were essentially similar, and the plateau length for both tubes operating together was 325 V.

The routine operating voltage was chosen as 1600 V, this being near the center of the plateau for each of the tubes. At this voltage the increase in the rate of counting for an increase of 100 V on the anode was 0.51%, 0.63%, and 0.64% of the rate of counting at 1600 V for the top tube, bottom tube, and both tubes together, respectively.
If the scaler input sensitivity was set at 0.25 V, the normal setting for Geiger tube operation, the plateaux became shorter (150-175 V), the operating voltage higher (1675 V), the slope greater (5.22-7.00%/100 V at 1675 V), and the number of counts recorded per unit time less, due to greater coincidence loss. The effect of altering the scaler input sensitivity is discussed in greater detail below.

Uniformity of counting efficiency across the diameter of the window.
The variation of the sensitivity of the counters at points on the windows at different distances from the anode wire was measured by observing the count rate of a small spot (0.15 in. diam.) of $^{14}$C glucose on a piece of chromatogram paper at intervals along a diameter of each window. As first constructed, the tubes were fitted with plain Mylar windows, and it was soon apparent that the efficiency across the windows of such tubes was by no means uniform (Fig. 6). If the glass bead on the anode wire was too close (0.1 in.) to the window (Fig. 6, a), there was a pronounced fall in sensitivity over a central area of about 0.25 in. diam., sensitivity also falling off more than 1 in. from the centre of the window. Raising the level of the glass bead to about 0.25 in. from the window removed the area of low sensitivity from the centre of the window (Fig. 6, b), but sensitivity fell off even more rapidly than before at distances greater than 1 in. from the centre. This lack of uniformity was corrected by fitting the tubes with windows made of Mylar but sputtered on both sides with very thin films of metallic gold (Fig. 6, c). The gold sputtering, by avoiding the insulating effect of plain Mylar windows, presumably enabled a more uniform electric field to be maintained across the counter windows. By using gold-sputtered windows the sensitivity was kept uniform as far as 1.75 in. from the centre of the window.
This uniformity was formally checked with the tubes mounted in position on the counter. Each tube was checked individually at 1/3 in. intervals along the diameter of the window, and both tubes were checked together (Fig. 7). The checking of each tube was performed with the high voltage supply to the anode of the opposing tube both on and off; no difference was observed. The separate tubes, and both tubes together, were all uniformly sensitive over an area of 1.75 in. radius. However, in order to allow for a possible incorrect alignment of chromatogram spots in the counting position, the maximum permissible area for mounting the spots on the loading machine is a circle of diam. 3 in., thus allowing for a margin of 0.25 in. error. The count rates for the test spot of 14C within 1.5 in. of the centres of the windows were as follows (25 measurements of 1 min. each across each window): top tube, 12425 ± 151 counts/min. (12176-12675); bottom tube, 12362 ± 168 counts/min. (12047-12611); both tubes together, 24711 ± 211 counts/min. (24372-24932). All the observed counts fell within 1.5 standard deviations of the corresponding mean value.

Changing cylinders of counting gas. The counter tubes continue to operate satisfactorily for about 4 min. after the cessation of the supply of counting gas (Fig. 8). This is sufficient time to permit changing a gas cylinder during a lengthy run without interrupting the counting operation.

Background levels. No shielding against background radiation is provided for the counter tubes. The background count rate with each tube is usually 140-150 counts/min. The reproducibility of the background count rate agrees with statistical expectation; i.e., the standard deviation is equal to the square root of the number of counts recorded. The following are some typical background count rates with both tubes together at three time intervals:
the background count for each period was repeated for 30 consecutive
intervals; 1 min. counts, 205 ± 15 counts/min. (168-243); 10 min. counts,
205 ± 3.8 counts/min. (197-211); 20 min. counts, 204 ± 3.3 counts/min.
(200-213).

With the tubes removed from their mounts and separated from each other,
the background of both tubes together is equal to the sum of the background
counts with each tube separately. However, when the tubes are mounted on the
assembly, one tube being directly above the other, the background count rate
of both tubes together is only about 70% of the sum of both tubes separately.
The explanation for this effect is probably that many background counts
originating from cosmic sources give rise to pulses produced simultaneously
in both tubes. Since the scaling circuit has a definite resolving time
(1 μsec.) only one of these pairs of pulses is recorded; presumably about
60% of the background counts are due to cosmic rays arriving from a
vertical direction, and of these 70% are not recorded. With the tubes
separated in space this anti-coincidence effect does not operate, and hence
the background count with both tubes operating together equals the sum of those
with the individual tubes.

Resolving time of the tubes; correction for coincidence losses. The
recovery time of the tubes was measured singly and together by determining
the count rates with samples of {14C} glucose of varying known absolute
activities spotted onto Whatman No. 1 filter paper. The apparent resolving time
of the tubes varied considerably as a result of altering the input sensitivity
voltage of the scaler. At an input sensitivity of 0.25 V (the normal value
used for Geiger-Müller tubes), the resolving time for each tube was found to
be about 285 μsec., a typical value for Geiger-Müller tubes. However, if
the input sensitivity of the scaler was set at 0.02 V, the resolving time for
each tube individually was found to be about 80 μsec., and for the two
tubes working together, 53 μsec.; the tubes were therefore not operating in
the Geiger region. By examining the shape of the output pulses of the tubes
with a cathode ray oscilloscope, it was found that at low counting rates,
permitting the tube to recover completely after each pulse, the tubes were
acting as true Geiger tubes. As the number of events occurring in each tube
per unit time increased, an ever greater number of events occurred in the
tube before recovery was complete. On such occasions a smaller (proportional)
pulse was produced, and with a low input sensitivity on the scaler, these
smaller pulses were recorded. As the time required for the tubes to recover
costently to be able to produce a proportional pulse was much less than that
for a Geiger pulse, the overall resolving time of the tubes was decreased.
Since the system was quite stable at scaler input sensitivity of 0.02 V, and
there was no increase in the rate of background counting, we have continued
to use the equipment in this way.

When the two tubes are used in parallel, twice as many events per unit
time occur in both tubes together as occur in each tube separately, but
the number of events occurring in each tube remains unaltered as a result
of the presence of the other tube. One would therefore expect that the
overall resolving time of the double tube system would be only half that
with either tube alone. However, with the two tubes in parallel a coincidence
loss is possible due to the finite resolving time of the scaler; this is not
the case with a single tube since the resolving time of the scaler is much
less than that of the counter tube. For this reason, the overall resolving
time of the two tubes in parallel is about 60% of that with a single tube, and
not 50%.
The low resolving time of the system, together with the use of
two tubes in parallel, has resulted in much smaller coincidence losses
than is normally observed with a single Geiger-Müller tube. For example,
at true count rates/min. of 10,000, 20,000, 50,000 and 100,000, the
coincidence losses are 0.97%, 1.71%, 4.22% and 8.11%, respectively, for
the present system, compared with 4.76%, 9.03%, 20.00% and 33.33%,
respectively, for a typical Geiger counter (resolving time 300 µsec.).

Efficiency of counting. With a $^{14}$C-labelled compound spotted onto
Whatman No. 4 filter paper lying between the two layers of the Mylar
strip, and counted simultaneously by two tubes fitted with gold-sputtered
Mylar windows, 10.63% of all disintegrations are recorded after
correction for coincidence loss and deduction of the background count.
The sensitivities of the two tubes did not differ by more than 1.5%.

Reproducibility of counting

Background counting. Consecutive background counts were made for
two periods of 3.5 - 4 days, each counting period being for 0.01 day (Fig. 9).
Each sequence commenced on Friday at noon, terminated at midnight on
Monday or noon on Tuesday, and thus encompassed both a full working day
and a weekend, in order to find whether the lessened demands on the
main power supply during the weekend and at night affected the background
counts. During the first 4 day period (Fig. 9 a) the Mylar tape was not
moved between each count; in the second period (3.5 days), run the following
weekend, the tape moved automatically between each count (Fig. 9 b).

For the counting sequence with the tape stationary, there was a
slight fall in the background count between about Sunday noon and
Monday noon; otherwise there was no consistent variation with time. Over
the 96 hr. period the mean value of the background count (400 observations)
was 242.3 ± 4.61 counts/min. (231-254); for groups of ten consecutive counts each, the mean varied between 237.4 and 247.6 counts/min., while the standard deviation varied between 22.5 and 26.5 counts/min., the average value being 24.1 counts/min. In the second sequence, with the tape moving between each count, there was more variation than before over long periods (e.g. from Saturday morning to Sunday morning), but such variation took some 12 hr. to reach a max. and a similar period to return to a min. value. The short term variation among consecutive counts was the same as with the stationary tape: for groups of ten consecutive counts each, the mean varied between 233.9 and 263.5 counts/min., while the standard deviation ranged from 12.2 to 16.7 counts/min., the average being 14.2 counts/min. Thus, the determination of a background count of 10-20 min. every 1 or 2 hr. during an automatic counting sequence is sufficient to provide a reliable measure of this value.

Counting of chromatogram spots. A comparison was made of the manual and automatic counting of 36 spots on the radiochromatogram shown in Plate 1. Manual counting for 1 min. intervals was performed without cutting the spots out of the paper. One side of the paper was counted at a time with a single Geiger-Müller tube of 2.25 in. internal diam. Individual index card masks were prepared for each of the 36 spots; as well as saving time, since each spot was to be counted several times, the preparation of individual masks probably increased accuracy compared with arranging loose cards to mask the unwanted areas. However, cutting out each mask required 1-2 min. With this procedure, counting all 36 spots on one side of the chromatogram took 1.75 hr.; the chromatogram was actually counted 8 times on each side. The background was deducted from each count, but the counts were not corrected for coincidence losses, since it
was the reproducibility which was required, not the absolute value. With the particular counter tube used, 7.7% of all disintegrations were recorded; a summary of the findings is reported in Table 1.

The spots were next excised from the chromatogram (30-60 sec./spot), and were loaded onto the Mylar tape. Owing to the sizes of some of the spots these had to be subdivided; in all a total of 72 counting positions was required, including 10 background positions. Once the spots were ready to be applied to the Mylar tape, the actual loading operation took less than 19 min. Three series of counts were made, each replicated 8 times to correspond with hand counting: (1) each counting position was counted for 60,000 sec.; (2) each position was counted for 600.00 sec., or 10,000 counts, whichever was less; (3) each position was counted for 1,200.0 sec., or 20,000 counts, whichever was less. These counts were corrected for coincidence losses, and background was deducted. The results are reported in Table 1.

Even with a similar counting period of 1 min., automatic counting is considerably more reliable than manual counting. This can probably be explained as being partly due to a more accurate location of the chromatogram spot in the counting position in the automatic system, and much higher reproducibility of the timing system in the automatic equipment. A 60 sec. mains frequency count with the automatic system (20 observations) gave a value of 3601.75 ± 0.447 cyc./min. (3601-3602); with the manual system, using an Eagle Signal Corp., Maline, Ill., U.S.A., timer, the mean (20 observations) was 3595 ± 22.0 cyc./min. (3549-3640). For routine use a program of 600.00 sec. or 10,000 counts, whichever is reached first, has been found to be satisfactory. Assuming a background rate of
210 counts/min., the standard deviation on even the lowest count (a background) would not exceed about 2% of the mean value.

**Cutting spots out of the chromatograms**

The use of the automatic counter necessitates the cutting of large numbers of spots out of chromatograms. A completely satisfactory and rapid method for doing this has not yet been devised. The available methods were tested by tracing ten irregularly shaped "chromatogram spots" onto each of three identical sheets of chromatography paper. The spots from each paper were cut out using three different methods by operators skilled in the use of each method. The time taken for each operator to cut out all ten spots under competitive conditions was noted. The results were as follows: (1) sharp scalpel, against a backing of blotting paper, 195 sec.; (2) "Vibrotool" (Burgess Vibrocrafters, Inc., Grayslake, Ill., U.S.A.) fitted with a pointed arrow-head tip, used against a backing of Transite, 202 sec.; (3) scissors, 255 sec. By common consent it was agreed that the neatest result was obtained with the "Vibrotool".
SUMMARY

1. A technique has been developed for the automatic measurement of radioactivity on areas of paper from paper chromatograms.

2. The positions of radioactive compounds on the chromatograms are determined by radioscintigraphy and the appropriate areas are cut out from the chromatogram.

3. The excised chromatogram spots are sandwiched between two continuous bands of thin plastic polyester film in an apparatus designed for this purpose.

4. In another apparatus, the accumulated double band of polyester film bearing the chromatogram spots is automatically passed in a discontinuous manner between two closely-opposed detector tubes. Each spot remains stationary between the detector tubes for a predetermined counting period. For each chromatogram spot the number of counts recorded, together with the elapsed time, is automatically printed onto paper tape. The double band of polyester film is then automatically advanced until the next spot lies between the detector tubes, and the cycle is repeated.

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EXPLANATION OF PLATES

Plate 1. Radiograph of the radiochromatogram used to investigate the reproducibility of manual and automatic counting. The radiograph (14 in. x 17 in.) was obtained with Kodak Single-Coated Blue-Sensitive Medical X-Ray Film, exposed for 3 days.

Plate 2. Apparatus for counting chromatogram spots between two continuous layers of Mylar film.

Plate 3. Equipment for counting chromatogram spots mounted between two continuous layers of Mylar film; general view.

Plate 4. Equipment for counting chromatogram spots mounted between two continuous layers of Mylar film; close-up showing details of detector tubes, rollers for alignment, and mounting of drive motor.
Chlorella
Photosynthesis with $^{14} \text{CO}_2$

Plate 1
Fig. 1. Diagrammatic representation of loading machine. Key: 1, supply spool of Mylar for bottom layer; 2, wooden table top faced with plastic; 3, illuminated window; 4, opposed rubber-covered rollers; 5, supply spool of Mylar for upper layer; 6, guide roller; 7, take-up spool; 8, felt pads; 9, supply spool of kraft paper; 10, machine for dispensing marker tape; 11, foot pedal for same; 12, motor for driving take-up spool; 13, foot switch for same. Scale = 1 ft.

Fig. 2. Diagrammatic representation of counter assembly. Key: 1, supply spool bearing kraft paper, and chromatogram spots between two layers of Mylar; 2, kraft paper; 3, double layer of Mylar bearing chromatogram spots; 4, guide rollers; 5, opposed detector tubes; 6, guide rollers; 7, take-up spool; 8, motor drive for take-up spool; 9, light for photocell; 10, photocell; 11, switch for selecting either or both detector tubes; 12, cables for connexion to scaler and high voltage supply; 13, gas inlet and outlet tubes; 14, knurled drive wheel for take-up spool. Scale = 1 ft.

Fig. 3. Section through detector tube showing details of mounting. Key: A, sapphire plug (U), with teflon insulator; B, threaded insert holding anode wire; C, knurled locking ring for threaded insert; D, gas inlet tube; E, gas outlet tube; F, pillar supporting anode; G, body of tube; H, anode wire (0.003 in. diam. tungsten); I, glass bead; J, flange soldered to tube body; K, distance between base plates is 0.063 in.; L, lower base plate; M, gold-sputtered Mylar windows; N, rubber O-ring (4.25 in. x 0.125 in.); O, six bolts holding flange to base plate; P, upper base plate. Scale = 1 in.
Fig. 4. Plan of detector tube counting. Key: A, Mylar band; B, base plate; C, outer diam. of flange on tube; D, outer diam. of tube; E, chromatogram spot no. 1 in counting position; F, max. permitted diam. of counting position; G, marker tape for chromatogram spot no. 1; H, photoelectric cell assembly; I, spacer strip (0.063 in. thick) separating base plates; J, marker tape for chromatogram spot no. 2; K, chromatogram spot no. 2 after counting. Mylar band moves from left to right. Scale = 1 in.

Fig. 5. Plateau of two detector tubes in parallel.

Fig. 6. Sensitivity of tubes across face of window under different conditions. A spot (0.15 in. diam.) containing $^{14}C$ on chromatogram paper was counted at different distances from the centre of the window. A, plain Mylar window, glass bead 0.1 in. from window; B, plain Mylar window, glass bead 0.25 in. from window; C, gold-sputtered Mylar window, glass bead 0.25 in. from window.

Fig. 7. Sensitivity of both tubes in parallel across face of gold-sputtered windows.

Fig. 8. Effect of turning off the gas supply on the count rate.

Fig. 9. Stability of the background count rate. Each point represents the background count rate for a period of 0.01 day. A, Mylar band stationary throughout; B, Mylar band moved 5 in. between counts.
Table 1. Comparison of counting reproducibility using a manual system, and an automatic system, at three different programme settings.

Thirty-six spots of compounds labelled with $^{14}$C on a two-dimensional paper chromatogram were counted manually for 1 min. periods, 8 times on each side of the paper. The spots were excised from the chromatogram and were automatically counted 6 times on each of three programme settings, both sides of the paper being counted simultaneously. The automatic results have been corrected for coincidence losses and background has been deducted; in the manual results coincidence correction has not been applied, but background has been deducted. The results for each technique are presented as the average standard deviation for the spots in question expressed as a percentage of the respective mean, together with the range of standard deviations found, again as percentages of the mean. The ranges of actual rates of counting are also presented, as well as a measure of the total $^{14}$C in the sum of all 36 spots.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Manual</th>
<th>Automatic</th>
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<tbody>
<tr>
<td>Program</td>
<td>60 sec.</td>
<td>50,000 sec.</td>
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<td></td>
<td>(average of both sides)</td>
<td>or</td>
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<tr>
<td>All 36 compounds</td>
<td>10,000 counts</td>
<td>20,000 counts</td>
</tr>
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</table>

| Average standard deviation (as % of means) | 2.23 | 1.78 | 1.02 | 0.74 |
| Range of standard deviations (as % of means) | 0.90 - 9.09 | 0.60 - 6.81 | 0.31 - 2.27 | 0.34 - 1.74 |

15 Most active compounds (together containing about 50% of all the \( ^{14} \mathrm{C} \))

| Range of activities (counts/min.) | 11340 - 13800 | 16900 - 27900 |
| Average standard deviation (as % of means) | 1.59 | 0.91 | 0.86 | 0.63 |
| Range of standard deviations (as % of means) | 1.20 - 2.04 | 0.60 - 1.43 | 0.39 - 1.40 | 0.34 - 1.00 |

21 Least active compounds (together containing about 5% of all the \( ^{14} \mathrm{C} \))

| Range of activities (counts/min.) | 417 - 11377 | 517 - 16293 |
| Average standard deviation (as % of means) | 2.69 | 2.40 | 1.14 | 0.81 |
| Range of standard deviations (as % of means) | 0.90 - 9.09 | 0.98 - 6.81 | 0.31 - 2.27 | 0.43 - 1.74 |

Total \( ^{14} \mathrm{C} \) in all 36 compounds (counts/min.)

| (8) | (9) | (6) | (6) |
| 586131±7592 | 944201±7269 | 936226±3985 | 940399±3323 |
| 570633±525919 | 932500±57317 | 926105±940372 | 937056±94488 |

0
Fig. 1
Fig. 5
Fig. 6
Fig. 7
Fig. 8

Time after turning off gas supply (min)

$10^{-3}$ x counts/min

MU-25926
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