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QUANTITATIVE RADIO PAPER CHROMATOGRAPHY

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The various techniques utilised in the preparation and measurement of a radio paper chromatogram are commonly referred to as quantitative radio paper chromatography. The scope and application of this technique, which combines paper chromatography and the use of radioactive tracers, have increased considerably during the past decade. This is primarily due to the fact that the measurement of the activity is performed directly by scanning the paper with an appropriate measuring device, which is now usually entirely automatic. Consequently, a rapid comparison of the activity of the separated substances is possible.

1. PREPARATION OF RADIOCHROMATOGRAMS

A radio paper chromatogram is obtained when radioactive substances are separated or when non-radioactive substances are separated and subsequently labelled, either with a radioactive reagent or by activation.

(*a*) *Chromatography of radioactive substances*

In the chromatography of radioactive substances, particular care and precautions must be taken over and above the usual procedures which chromatography involves.

Because of the high sensitivity with which the radio isotopes can be detected and the chemical non-specificity of this detection, it is necessary to take the utmost care to avoid contamination.

The radioactive solution should preferably come into contact only with glass, since certain radioactive ions partially attach themselves to metallic parts, such as

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the needle of the micro-syringe with which the solution is placed on the paper, and are subsequently liberated by ion exchange. For example, in the chromatography of a solution of ^{14}C-labelled substances containing phosphate ions, the authors observed a spurious radioactive spot on the chromatogram, which was identified as ^{32}P-phosphate. This originated from ion exchange in the needle of a syringe that had previously been used for a solution containing ^{32}P-phosphate ions; evidently washing with water had not been sufficient to eliminate this contamination.

In the chromatography of a substance labelled with radioactive isotopes emitting soft beta rays (^{14}C, ^{35}S, etc.) it is necessary to keep in mind that the self-absorption of this radiation may not be uniform, either because of irregularity in the thickness of the paper or because of irregularity in the distribution of the substance through the paper.

WINTERINGHAM, HARRISON AND BRIDGES[1] have found that the irregularity in the thickness of a sheet of Whatman No. 1 paper is about 4 %. In chromatograms containing substances labelled with ^{14}C or ^{35}S, this irregularity produces an error of some 2 or 3 % in the value of the radioactivity.

Another significant error that may occur in some cases arises from the irregular distribution of the substance within the paper, caused by the method used for the evaporation of the sample to be chromatographed. It is known that in chromatography, evaporation of the successive drops of the solution can be speeded up by applying a stream of hot air from a standard hair dryer. This stream of hot air causes a partial accumulation of the substance on the lower surface of the sheet, because evaporation is more rapid there. This results in a non-homogeneous distribution of the substance through the paper. The authors have found[2] that, owing to this accumulation on the lower surface (R) of the paper, the amount of radioactivity measured on the upper surface of the paper (F) is decreased, while that on the lower surface (R) is increased (Table 1). Such irregularities in distribution will gradually be eliminated

TABLE 1

EFFECT OF VARIOUS CONDITIONS OF EVAPORATION ON THE MEASURABLE ACTIVITY OF ^{14}C-GLUCOSE ABSORBED ON WHATMAN NO. 1 PAPER

The measurements, made with a Geiger counter on the upper (F) and the lower (R) surfaces of the sheet, are expressed in c.p.m. and are the means ± s.e.m. of 8 samples of 40 μl each, applied 2 μl at a time. The current of air was directed at the lower surface of the sheet.

Evaporation conditions	*F*	*R*	*Mean*	*R/F*	*R'/F'**
Without current of air	3135 ± 45	3045 ± 45	3095 ± 35	0.97 ± 0.01	0.96 ± 0.01
With current of cold air at 50 cm	2350 ± 30	3970 ± 75	3160 ± 30	1.69 ± 0.05	0.98 ± 0.01
With current of cold air at 5 cm	2025 ± 35	5415 ± 35	3720 ± 25	2.67 ± 0.05	0.97 ± 0.01
With current of hot air at 5 cm	1995 ± 25	5745 ± 35	3870 ± 35	2.88 ± 0.05	0.97 ± 0.01

* R'/F' is the ratio of the activities measured after development of the chromatogram (according to POCCHIARI AND ROSSI[2]).

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during further chromatographic development, and it is therefore only a source of error in the measurement of the radioactivity of the substances that remain in the initial position ($R_F = 0$). A similar error occurs if a comparison is required of the activity of the sample before chromatographic development with that of the different substances separated on the chromatogram after development. To eliminate the errors resulting from irregularity in the distribution of the substance, it is necessary to take as the true value of the activity the mean of the values measured on both sides of the paper; if this ratio is greater than 1.5, a correction factor must be applied[2].

(b) *Labelling of separated substances with a radioactive reagent or by activation*

Up to the present these methods of labelling have had limited application, and they are mentioned here only on account of their originality and because they are applicable in the case of special problems.

In order to measure certain amino acids directly on chromatograms, WINTERINGHAM *et al.*[1] exposed the chromatograms to the vapours of ^{131}I methyl iodide. They found that the amount of the radioactivity remaining on the radiochromatograms after pumping away the excess of methyl iodide was proportional to the quantity of amino acids present. In order to estimate substances containing bromine and chlorine, the same authors[1] activated the latter by irradiating the chromatogram with neutrons in an atomic pile.

An interesting method, which permits the application of radio paper chromatography to a substance that has been labelled with a stable isotope, was developed by FÖGELSTROM-FINEMAN *et al.*[3]. They were able to detect a substance labelled with ^{18}O by activating the substance with protons, whereby ^{18}O is transformed into radioactive ^{18}F. Since the paper contains oxygen, which normally has a content of 0.2% of ^{18}O, it is necessary to transfer the separated substances to a metal sheet before activation. This can be done by eluting the spots with a special device in a direction perpendicular to the strip, so that the relative positions of the spots are not altered.

2. MEASUREMENT OF RADIOCHROMATOGRAMS

(a) *Measuring devices*

An approximate evaluation of the radioactivity of the spots on a chromatogram can be made by autoradiography (FINK, DENT AND FINK[4]). The radiochromatogram is brought into contact with an X-ray film for a sufficient length of time to produce darkened areas on the film after photographic development. To obtain an optical density of 0.6 with the β-rays of ^{14}C, it is sufficient that about 10–20 million particles per cm^2 bombard the emulsion during the entire period of exposure. Some examples of autoradiography are shown in Figs. 11, 12 and 13.

On the other hand, quantitative determination of the radioactivity present in a radiochromatogram may be achieved by direct scanning of the paper with measuring

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devices, such as an end-window Geiger counter*, a flow counter**, an ionization chamber or a scintillation counter. The proper choice of one of these devices is dependent on the type of radiation to be measured and the degree of efficiency desired.

TOMARELLI AND FLOREY[11] were the first to construct a simple apparatus for scanning strips of paper. These were mounted on a sliding base, which was moved manually at regular intervals across a Geiger counter. The movement of the chromatogram, the control of the measuring period and the recording of the number of impulses can be done effectively by hand. Devices of similar types have been used by numerous investigators; an example is shown in Fig. 1.

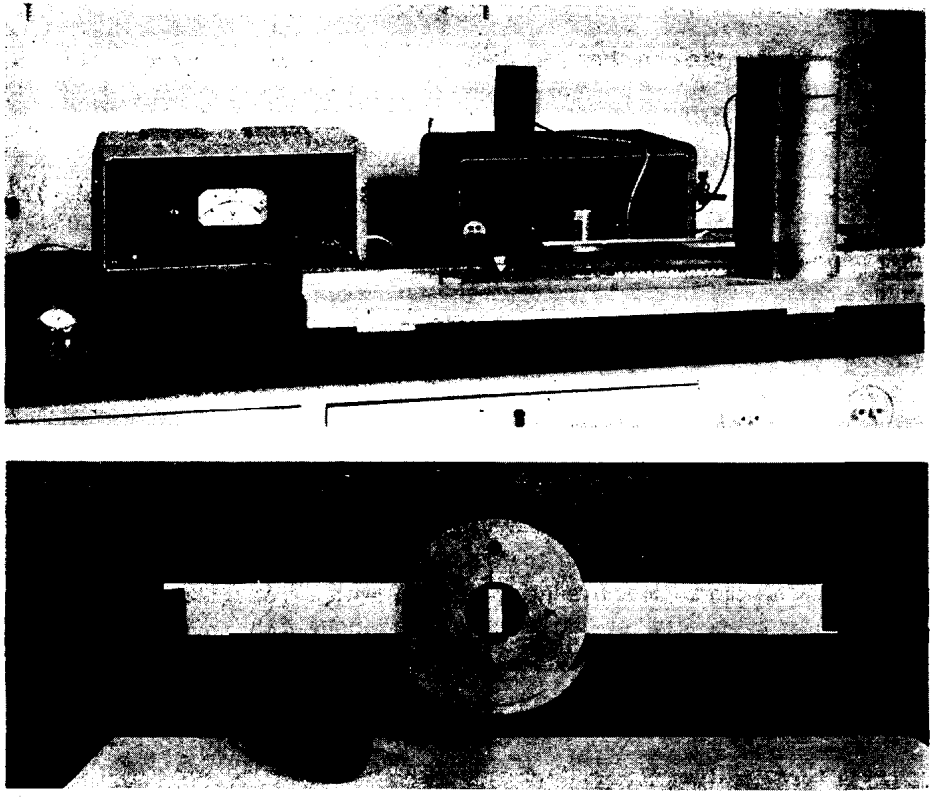

Fig. 1. Apparatus for manual measurement of monodimensional radiochromatograms. (a). General view. (b). Detail showing the slide which carries the chromatogram and the scanning slit (view from above after removing the lead shield and the Geiger counter).

* FULLER[5] has described a counter with an end-window of Mylar, 0.5 thousandths of an inch thick. This window has a lower absorption than one of mica and thus permits the measurement of ^{14}C with an absolute efficiency of about 10%.

** Flow counters have been suitably adapted for the scanning of radiochromatograms. Some investigators have made the contact between the counter and the chromatogram as airtight as possible (GRAY *et al.*[6]; BANGHAM[7]; LÖWENSTEIN AND COHEN[8]; OSINSKI[51]). DEMOREST AND BASKIN[9], on the other hand, have modified the counter by incorporating into it a chamber with a sliding base carrying the chromatogram. A similar counter without a window but completely airtight has been constructed by HARRISON AND WINTERINGHAM[10]. This counter has a geometrical efficiency of 4π.

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It is evident that although the measurement of a radiochromatogram with such a simple apparatus is accurate, it is inconvenient because of the considerable amount of work and time involved. Various ways of simplifying this technique have therefore been suggested.

The most simple method is localization of the spots by autoradiography, followed by quantitative determination of their activity. Such measurement is improved by the use of a counter larger than those usually employed, whereby it is possible to determine the activity of an entire spot in only one measurement (see, *e.g.*, KATZ AND CHAIKOFF[12] and STEENBERG AND BENSON[13]). In another method all the zones of the chromatogram are measured simultaneously with a multiple measuring device. GILBERT AND KEERNE[14] have constructed such an apparatus containing 30 adjoining counters each with a rectangular window 8 × 25 mm. The impulses transmitted by these counters are accumulated in a series of condensers. The potential of each of these condensers, which is determined with an electrometer at the end of the measuring period, gives the value of the activity present in the corresponding zone of the chromatogram.

In measuring the activity of radiochromatograms containing isotopes emitting soft radiation (^{3}H, ^{14}C, ^{35}S), the efficiency in the measurement can be increased, and thus the counting time reduced, by elimination of the self-absorption of the radiation from the paper. This has been achieved by ROUCAYROL, OBERHAUSEN AND SCHULER[15] who impregnated the chromatogram with a solution of phenyl-biphenyl-oxadiazole in toluene and afterwards measured the light coming from the paper with a multiplier phototube. Recently, FUNT AND HETHERINGTON[16] have found that the use of a solvent less volatile than toluene, such as monoisopropylbiphenyl, guarantees a better reproducibility of the measurements. SELIGER AND AGRANOFF[17], using a solution of anthracene in toluene, carried out the measurements after evaporation of the solvent.

However, the method most often applied to reduce the manual work involved has been the automatic advancement of the chromatogram and automatic recording of the radioactivity.

(*b*) *Automatic apparatuses*

Up to the present about 30 such apparatuses have been described; these can be divided into two groups according to the system used for advancement of the chromatogram and for recording the radioactivity of the spots. The type of recording can be graphic or numerical, and the mode of advancement of the chromatogram can be continuous or discontinuous, but for practical purposes the continuous advancement of the chromatogram is generally combined with graphic recording and the discontinuous method with numerical recording.

(1) *Apparatuses with continuous advancement of the chromatogram and graphic recording of the radioactivity*

With apparatuses of this type, the statistical sequence of impulses coming from the

counter is transformed by a ratemeter into a continuous flow of current which drives a graphic recorder*.

The first such devices were described in 1951 by MÜLLER AND WISE[19], WILLIAMS AND SMITH[20] and FRIERSON AND JONES[21].

Fig. 2 shows, for example, the plan of the apparatus used by MÜLLER AND WISE, together with a typical recording obtained. The activity of each spot is calculated

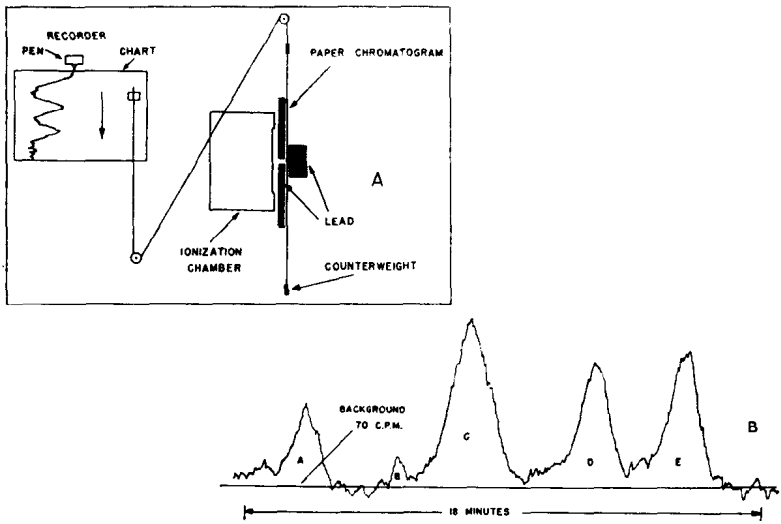

Fig. 2. (A). Scheme of the arrangement for the automatic advancement of a monodimensional radiochromatogram. (B). A typical record obtained with this apparatus. (According to MÜLLER AND WISE[19].)

by measuring the area enclosed between the corresponding peak and the base line; the error in this calculation is about 1 %.

Other devices based on the same general principle, but with small differences in the system of advancement of the chromatogram, have successively been described by various authors (JONES[22]; BRADLEY[23]; CARLESON[24]; LERCH AND NEUKOMM[25]; DEMOREST AND BASKIN[9]; PINAJIAN AND CHRISTIAN[26]; COHN, BUCKALOO AND CARTER[27]; STERNBERG[28]; VIGNE AND LISSITZKY[29]). Devices of this type have also been produced commercially for the past few years.

The factors that influence the profile of a curve are: the size of the slit; the speed of advancement of the chromatogram and of the recording paper; the electrical and mechanical inertia of the ratemeter and the recorder; and the statistical fluctuation of the radioactivity (BONET-MAURY[30]). These variables are strictly correlated. For example, WILLIAMS AND SMITH[20] have shown that in order to obtain an accurate recording, the slit width (S), the time constant (T) of the ratemeter and the speed (V)

* SOLOWAY, RENNIE AND STETTEN[18] have described a device in which the graphic registration, instead of being a continuous curve, is a series of parallel segments, their height being proportional to the number of impulses counted during a pre-set time interval of integration.

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of movement of the chromatogram should conform to the relationship $S = 4\ TV$.

A drawback to the system of graphic recording is that the degree of sensitivity of the instrument has to be selected according to the most active zone. This obviously causes a relatively greater percentage of error in the zone with the weakest radioactivity. In order to eliminate this factor, BERTHET[31] devised an apparatus which changes the degree of sensitivity of the recorder automatically when the radioactivity reaches pre-set values. By this means the error of the apparatus is kept constant and, for radioactivity varying between 300–30,000 c.p.m., is always under 2 %.

The areas on the graphic recording corresponding to the radioactivity of the spots on the chromatogram are generally measured with a planimeter. ROBERTS AND CARLETON[32] obtained under specific experimental conditions a proportional relationship between the height of the peak of the curve and the radioactivity of the whole spot. To achieve this, however, it was necessary to chromatograph, besides the sample, a standard series of the same substances. DURRUM AND GILFORD[33] avoided using a planimeter for the integration by making a device whereby registration of the integral curve is obtained. The activity of the spot is measured by making two successive chromatographic scannings; the first registers the radioactivity of each point and the second recording gives the integral curve, the two curves being superimposed. The difference between the ordinates of the second graphic recording drawn at the minima at each side of a peak of the first recording, gives a direct indication of the total radioactivity of the corresponding spot (Fig. 3). A similar apparatus has been constructed by SMILJANIČ AND RABUZIN[52].

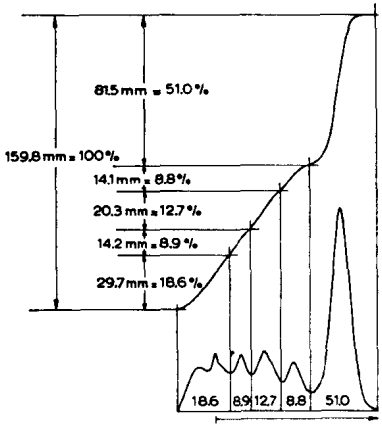

Fig. 3. Recorded curve of radioactivity of a scanned radiochromatogram and the corresponding integral curve (according to DURRUM AND GILFORD[33]).

Two automatic graphic devices described by WINGO[34] and ARONOFF[35] can be included in this group. These apparatuses, however, instead of recording one measurement, make a facsimile (Fig. 4) of the distribution of the radioactive spots on the chromatogram, the result being similar to an autoradiograph but obtained in a much shorter time. The device described by WINGO consists of a rotating cylinder on whose

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wall the chromatogram and the recording paper are wrapped next to each other. The impulses from the Geiger counter are fed through a scaler. This has a relay which controls the pen-recorder so that each impulse is registered. A succession of scannings produces a series of parallel recordings, which constitute a facsimile of the radioactive spots. In the apparatus of ARONOFF, on the other hand, the Geiger counter and pen-recorder move continuously in a direction parallel to the axis of the cylinder, which rotates continuously carrying the chromatogram and recording paper with it.

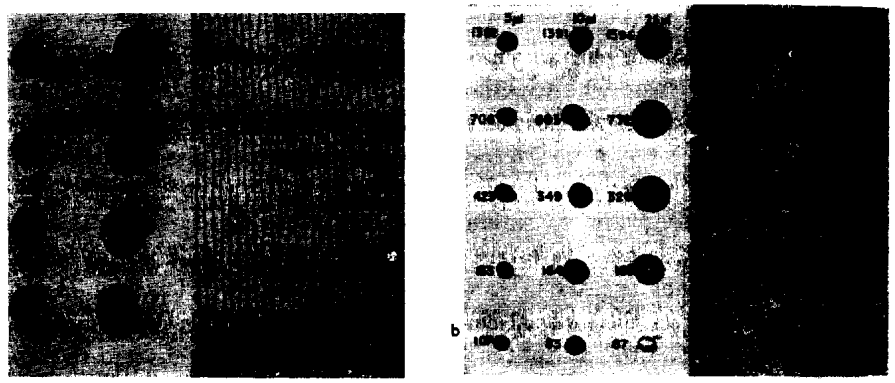

Fig. 4. Autoradiograph together with the corresponding facsimile record of two artificial chromatograms (a. according to WINGO[34], b. according to ARONOFF[35]).

The impulses from the counter, suitably modified, pass through the stylus and are registered on the sensitive paper at the point of contact. Thus, a combination of the two movements, namely the rotation of the drum and the advancement of the counter and pen-recorder, provides a complete scanning of the chromatogram. The tracing that appears on the paper is a facsimile of the radioactive spots, in the form of clusters of points.

(2) *Apparatuses with discontinuous movement of the chromatogram and numerical recording of the radioactivity**

Some apparatuses of this type record the number of impulses counted in a pre-set time period, while others record the time required to reach a pre-fixed number of impulses. ROCKLAND, LIEBERMAN AND DUNN[37] were the first to make an apparatus with discontinuous advancement of the radiochromatogram. The sliding platform with the chromatogram is moved in increments under an end-window Geiger counter by means of an advancing device. This latter comprises an interchangeable ratchet and slit, a spring and an electro-magnet. The movement of the sliding platform (with the attached chromatogram) is regulated by the dimensions of the ratchet (paired with the slit). The latter are interchangeable and can vary the increment from $^1/_{16}$ in. to 1 in. The impulses coming from the counter are accumulated on a binary scale. When

* WINTERINGHAM *et al.*[1] and PIPER AND ARNSTEIN[36] have described apparatuses with a system of graphic recording and discontinuous advancement of the chromatogram.

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the pre-set number of impulses is reached, the scaler (by means of a suitable circuit) transmits an electric impulse to a timer which registers the elapsed time. Immediately afterwards, the scaler and time marker are reset and the chromatogram is advanced to the next position. The value of the activity of each zone scanned can easily be obtained from the recording time. Such a system of counting with a pre-set number of impulses has, however, the disadvantage that much of the time required for scanning the entire chromatogram is spent in measuring only the background.

On the other hand, recording the number of impulses counted in a pre-set time presents a major construction difficulty, in that the mechanical recorder lacks the rapidity necessary to accumulate the statistical impulses coming from a highly active zone of a chromatogram.

HELLSTRÖM[38] has solved this problem by means of a "robot scaler" in which the impulses are accumulated in a series of decadic tubes and the recording is done at the end of each measuring period by means of an electromechanical read-out and a digital printing unit. The chromatographic strips to be scanned can be joined together, end to end, in one long strip with a maximum length of 40 m (Fig. 5). The strip moves

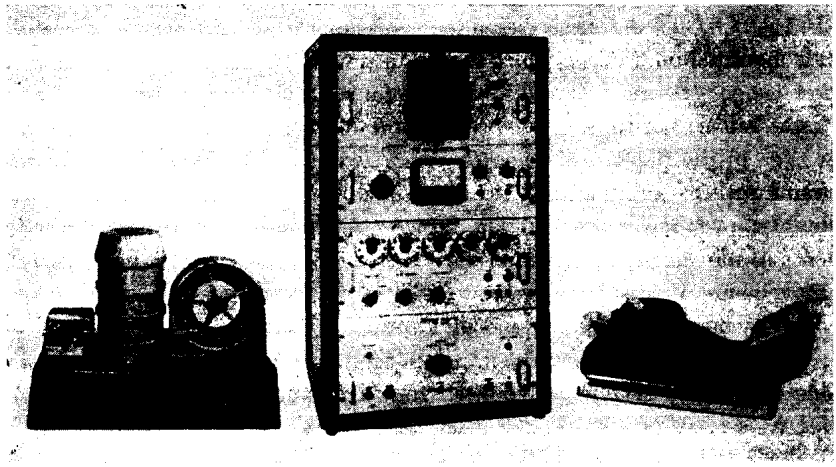

Fig. 5. Robot scaling equipment with chromatogram scanner, robot scaler and digital printing unit (according to HELLSTRÖM[38]).

step-wise under the Geiger tube of the scanning device, the step length varying between 3 and 7 mm. The impulses coming from the counter are counted by a decadic scaler and after a pre-set time interval the digits indicated on the decadic tubes are transferred by a read-out and a series of solenoid-operated plugs to the keys of an adding machine, which prints the digits of counted pulses on a strip of paper. Immediately afterwards the decadic tubes become reset and the chromatogram is automatically advanced to the next position.

The instruments discussed thus far (with the exception of that described in a short communication by PIPER AND ARNSTEIN[36]) can only scan strips of paper. Therefore, bidimensional chromatograms have to be cut into strips for measurement. With

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this method it often happens that the most radioactive zone of the spot is situated at the edge of the paper strip. It is, therefore, necessary that all points be measured with the same efficiency. This can be done by keeping the width of the strips to about 1 cm, which is less than that of the usual (3 cm) monodimensional radiochromatograms. In measuring the latter, less efficiency is in fact permissible along the edges of the strips, because the spots occur along the central axis of the chromatogram. If the width of the strip is reduced, even a better resolving power will be achieved. If the width is the same as the distance of advancement, the zone to be scanned will be a square and, therefore, the resolving power will be the same in both directions. In any event, measurement of bidimensional chromatograms by the above-mentioned system involves considerable handling. In the devices with graphic registration furthermore, when the adjacent graphs corresponding to each strip are assembled, the map obtained is expanded in one direction and consequently the distribution of the spots is less evident.

From all that has been said above, it is evident that to obtain the best results a map of the radioactive distribution of a bidimensional radiochromatogram should be made, and this involves designing a device specifically for that purpose.

In 1956 CHAIN *et al.*[39] described a series of apparatuses of this type. The chromatogram is mounted on a frame which moves in a vertical plane between two mica end-window Geiger counters, having a diaphragm with an open square 1 cm by 1 cm; the frame moves horizontally at pre-set time intervals. The scanning begins at the lower edge of the paper and when the whole width of the paper has been scanned, the frame returns automatically to the original position and at the same time the chromatogram is lowered 1 cm. The horizontal scanning commences again and the sequence of movements is repeated until the whole chromatogram has been scanned. The movements of the frame are controlled by an electromechanical timer (which is activated by means of a motor) and by a series of microswitches. These devices have different methods of recording the impulses coming from the Geiger counter: a photographic system, a system of counter stamping and an electronic system. *Photgraphic system*: In this method the impulses coming from the counter, after being divided in a scaler, are counted in a mechanical register (Fig. 6). The numbers appearing are automatically photographed at the end of each scanning period. To obtain a map of the numbers representing the distribution of the radioactivity on a bidimensional radiochromatogram, it is necessary to cut the photographic film into strips corresponding to each horizontal scanning and place these parallel to each other. The manual work involved in this operation can be avoided by using a stamping counter moved by a pantograph. *Stamping counter system*: The impulses are counted by a special mechanical stamping register (UGOLINI[40]), which is moved by means of a pantograph synchronized with the movement of the frame (Fig. 7). At the end of each measuring period, the number of impulses are reproduced on a sheet of paper in positions corresponding to those of the scanned zone of the radiochromatogram. It must be borne in mind, however, that the stamping counter of necessity has a mechanical inertia greater than that of the usual counter, and therefore the coincidence

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loss increases rapidly if the necessary counting rate is to be maintained to count some hundreds of impulses per minute (CHAIN *et al.*[39]). On the other hand, it is evidently not possible to augment the scaling factor noticeably, because then even the missed

Fig. 6. Automatic apparatus for the measurement of bidimensional radiochromatograms with photographic recorder (according to CHAIN *et al.*[39]).

remainder increases and hence the error in the measurement of the zone with the least radioactivity becomes excessive. An *electronic system* in which the afore-mentioned disadvantage is eliminated was finally adopted by these investigators[39]. The impulses are counted on a scale comprising a series of decadic tubes, and at the end of each measurement the numbers indicated on each tube are transferred by a completely electronic read-out unit (FRANK[41]) and a series of solenoid-operated plugs to the keys of an

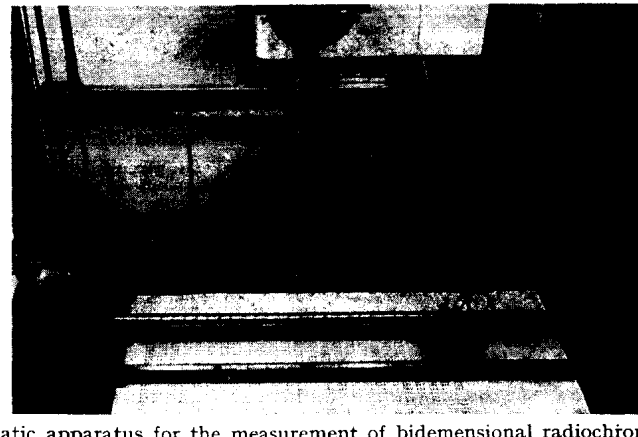

Fig. 7. Automatic apparatus for the measurement of bidemensional radiochromatograms with stamping recorder (according to CHAIN *et al.*[39]).

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electric typewriter whose carriage advances and rotates in synchronization with the horizontal and vertical movement of the frame carrying the chromatogram. As with the system of the mechanical stamping register, the number of impulses recorded by the typewriter corresponds in value and in position to the zone scanned on the chromatogram. Fig. 8 shows the numerical map thus obtained with a bidimensional radio-

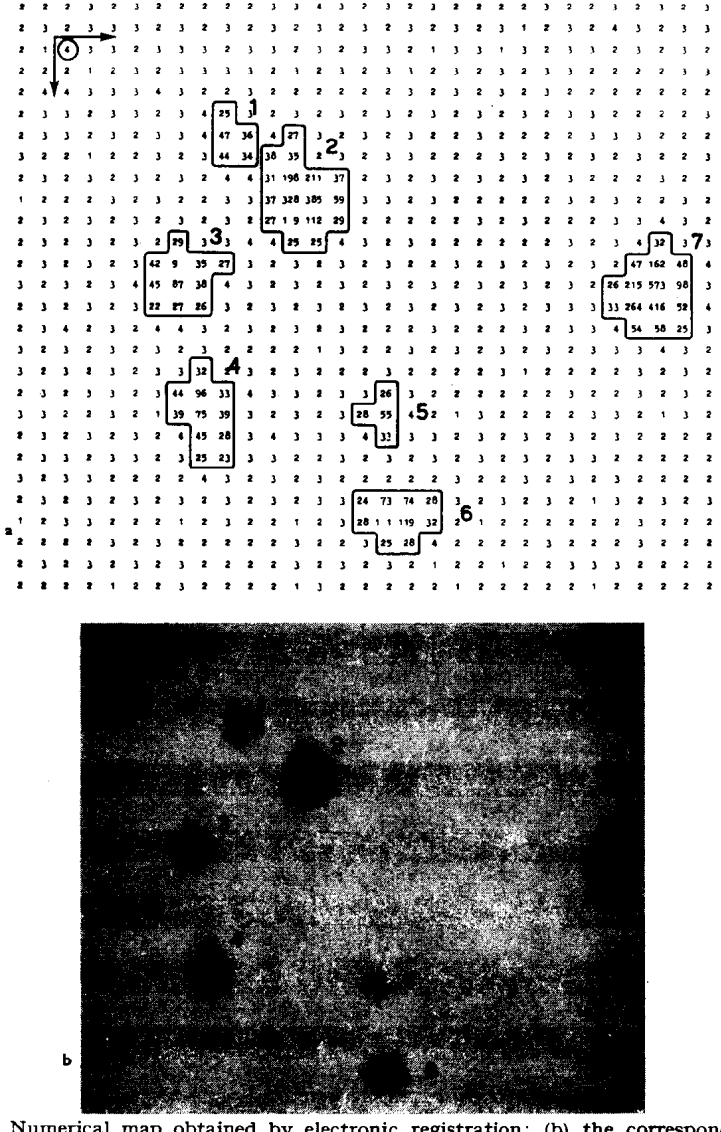

Fig. 8. (a) Numerical map obtained by electronic registration; (b) the corresponding radioautograph. In order to avoid the appearance on the map of figures with many zeros preceding the final digit such as 001, 002 etc., which would obscure the localisation of the active zones, the pulse from the read-out corresponding to the digit 0 is diverted to the solenoid of the advancement key. (According to CHAIN *et al.*[39]).

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chromatogram and, for comparison, also the autoradiograph of this example. It should be noted that the numerical map is obtained in 18 hours, whereas the autoradiograph requires 170 hours. With these devices CHAIN *et al.*[39] obtained an optimum reproducibility in the measurement of radioactivity present on a radiochromatogram. In fact, the standard deviation, even including the errors due to the apparatus, was found to be only slightly greater than that occurring with the statistical fluctuations of the radioactivity, which is equivalent to the square root of the number of impulses counted (Table 2, line 1). Furthermore, it has been shown that the mode of mounting the chromatogram does not influence the value found for the total radioactivity of each spot (Table 2, line 2).

TABLE 2

REPRODUCIBILITY OF THE MEASUREMENTS OF A RADIOCHROMATOGRAM CONTAINING SPOTS OF DIFFERENT INTENSITIES*

The radioactive spots are those of a radiochromatogram similar to that of Fig. 8. The results are expressed as c.p.m. ± standard deviation observed and, in parentheses, standard deviation calculated.

Spot	1	2	3	4	5	6	7
1. c.p.m. ± s.d. (mean of 8 expts.)	2190 ± 119 (47)	480 ± 40 (22)	1120 ± 79 (34)	490 ± 40 (22)	8870 ± 475 (94)	950 ± 53 (31)	1630 ± 79 (40)
2. c.p.m. ± s.d. (mean of 4 expts.)	2200 ± 132 (47)	460 ± 53 (22)	1110 ± 79 (34)	490 ± 40 (22)	8900 ± 449 (94)	950 ± 53 (31)	1600 ± 66 (40)

* According to CHAIN *et al.*[39].

Recently, an apparatus has been described by FRANK, CHAIN, POCCHIARI AND ROSSI[42], which is mechanically much simpler than the foregoing, in that the electromechanical devices for the horizontal and vertical movements of the radiochromatogram are eliminated. The radiochromatogram is simply attached by adhesive tape to one edge of a blank sheet of paper which is inserted into the carriage of an electric typewriter (Fig. 9). The chromatogram is then moved between the two counters by the automatic movement of the carriage. The decadic scale, the autoprogramme unit and the electronic read-out are the same as described heretofore[39,41]. The authors have also designed a similar apparatus for measuring monodimensional chromatograms. The chromatogram is mounted on a small frame joined at one end to the recording paper of an adding machine and at the other end to a counter balance (Fig. 10). After the number of pulses counted in each zone has been recorded, the recording paper advances and moves the chromatogram between the two counters. The scanning window is 5 × 30 mm.

All the apparatuses described above[39,42] also contain a device for automatic selection between two pre-set counting periods, one a multiple of the other. If, during the measurement of the radioactive zone of the chromatogram the number of impulses counted in a given time t (usually 45 seconds) is less than the pre-set threshold, this is considered as the background, and the apparatus moves on to scan the next zone. If, on the other hand, the number of pulses counted exceeds the value of the threshold, this indicates that the zone being scanned is radioactive, and the device continues

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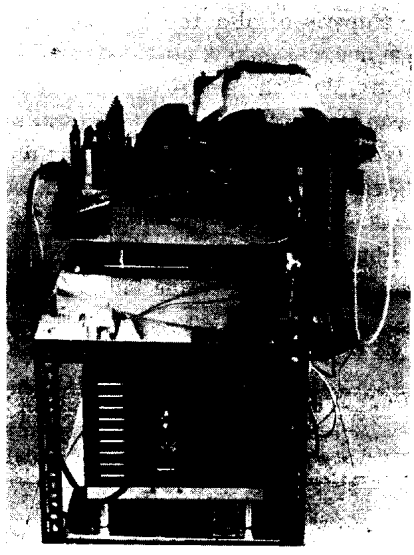

Fig. 9. Automatic apparatus for the measurement of bidimensional radiochromatograms, in which an electrical typewriter is used both for moving the chromatogram and for recording the pulses (according to FRANK *et al.*[42]).

to measure this zone for a period T equal to a pre-set multiple (usually 5 times) of the t time period. This autoprogramme is necessary for measuring bidimensional chromatograms, because in these the radioactive zones *in toto* represent only a small percentage of the entire area scanned.

The above-mentioned devices[39,42] are generally used for measuring different radioactive isotopes emitting β particles. The suitability of the apparatus depends mainly on the type of measuring device used (as already discussed on pp. 4 and 5), and on the collimation system. With regard to the latter, it should be remembered that when using ^{32}P it has been found[42] that the error in collimation exceeds that observed with radioactive isotopes emitting less penetrating rays, as for example ^{14}C

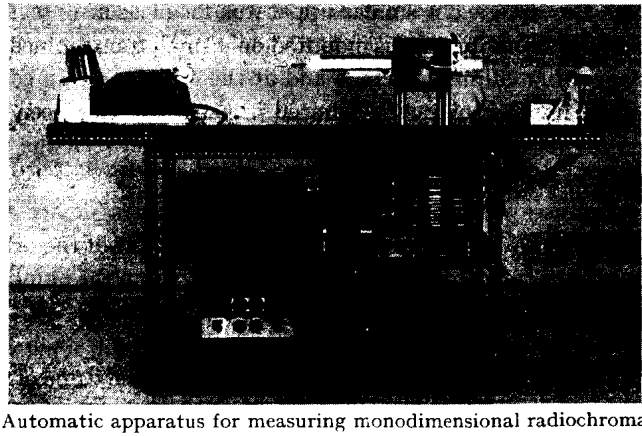

Fig. 10. Automatic apparatus for measuring monodimensional radiochromatograms.

References p. 17.

and ^{35}S. While the total relative intensity of the spots is not much altered by this error, it does cause an apparent enlargement on the numerical map and hence, a lower resolving power.

(c) *Use of the various measuring techniques in radio paper chromatography*

Up to the present radio paper chromatography has been utilized mainly in the field of biochemistry. In fact, in the study of intermediate metabolism with labelled substances it has provided a complete picture of the distribution of the tracer in the various metabolites. Such a picture can be obtained semiquantitatively by autoradiography. Examples are shown in Figs. 11, 12 and 13. Fig. 11 shows the autoradiograph of a

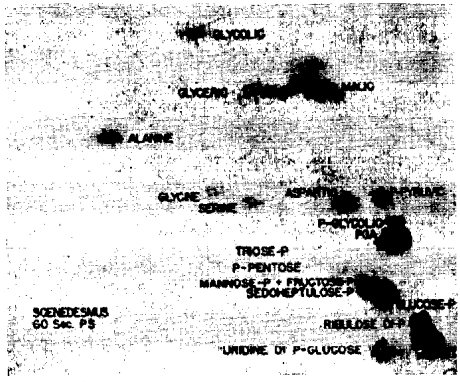

Fig. 11. Autoradiograph of a bidimensional chromatogram of an 80% alcoholic extract of *Scenedesmus* after 60 sec photosynthesis in radioactive CO_2 (according to CALVIN[44]).

bidimensional chromatogram of an alcoholic extract of *Scenedesmus alga* kept for 60 seconds in air containing $^{14}CO_2$. Identification of the various intermediate metabolites together with a kinetic study of the incorporation of ^{14}C into these compounds enabled CALVIN and his co-workers to suggest a cycle for the reduction of carbon in photosynthesis and to connect this cycle to other metabolic pathways (for bibliography see refs.[43,44]). Figs. 12 and 13 show autoradiographs of chromatograms of aqueous ethanolic extracts of rat diaphragm (BELOFF-CHAIN *et al.*[45]) and of rat brain (BELOFF-CHAIN *et al.*[46]) after incubation with uniformly-labelled ^{14}C-glucose. Such autoradiographs reveal that there is a distinct difference in the metabolism of glucose in the two tissues; in the muscle tissue the ^{14}C-labelled glucose was converted into oligo- and polysaccarides and in the brain into amino acids; in both instances glucose is also converted into lactate.

In order to determine a balance in the distribution of the tracer in the various metabolites, it is necessary to measure exactly the activity of a single spot. For this purpose, apparatuses for the manual advancement of the chromatogram and recording of the values were used by BELOFF-CHAIN *et al.*[45], who were able to study the effect of insulin on glucose metabolism in the isolated diaphragm of a rat. Considerable time and labour were required to accomplish such a study by this method.

References p. 17.