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THE CHROMATOGRAPHY OF RADIOACTIVE SUBSTANCES

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THE CHROMATOGRAPHY OF RADIOACTIVE SUBSTANCES

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CHAPTER

THE CHROMATOGRAPHY OF RADIOACTIVE SUBSTANCES

The chromatography of radioactive substances involves to some extent different conceptions from those used when unlabelled compounds are chromatographed. In the latter instance, it is frequently sufficient to be able to separate from each other only those compounds which are to be detected by the colour reagent used. The presence of other materials not affected by the location reagent is immaterial so long as they do not interfere with the particular compounds being investigated. If, for example, a transaminase reaction is being investigated by the formation of a particular aminoacid from the corresponding ketoacid, chromatographic analysis of the products of the reaction can ignore the presence of residual ketoacid because the ninhydrin or other reagent used to detect the aminoacid will not detect ketoacid. Thus, even if the aminoacid possesses chromatographic parameters identical with a ketoacid, the aminoacid can be separately distinguished.

When radioactive substances are used, however, this line of reasoning no longer holds. It becomes necessary to separate the compounds in which one is interested from <u>all</u> other compounds containing radioactivity, as techniques used to locate and assay radioactivity cannot distinguish between, say, C^{14} in aminoacid or in a ketoacid. Thus, care must be taken as to the constituents of the mixture to be chromatographed, and if radiochemical impurities cannot be avoided then a solvent system must be chosen such that there will be no serious interference from unwanted contaminants. In this chapter an account will be given of the more important methods for detection and assay of radioactive materials on paper chromatograms, while the following chapter contains a description of the application of these techniques

to the problem of the analysis of cell extracts following the incubation of biological systems with radioactive substrates.

HANDLING RADIOACTIVE MATERIALS

Space will not allow a detailed discussion of the theoretical aspects of ionizing radiation and of radioactive isotopes. It is suggested that workers new to the use of isotopes should read one of the many excellent books on the subject.[1,2,3] Nevertheless, there are some points regarding the manipulation and storage of these materials which deserve mention here. Radioactive isotopes decay to half their original activity at any given time in a period, which is characteristic for each isotope, known as the half-life. Of the isotopes most commonly used in biological research, C^{14} and Cl^{36} have such long half-lives (about 5,600 years and $4 \cdot 4 \times 10^5$ years, respectively) that decay need not be taken into consideration. Tritium (H^3) has a half-life of over 12 years, and unless a particular experiment is likely to run for a significant fraction of this period, the half-life can be ignored. With three other isotopes, however, Br^{82}, I^{131}, and P^{32}, the half-life is so short (36 hr., 8 days, and 14 days, respectively) that it becomes an important factor when these nuclides are employed. Thus, Br^{82}, I^{131}, and P^{32} must be used soon after preparation, or little of the original activity will remain. When Fe^{59}, Hg^{203}, S^{35} and Zn^{65} are to be studied (with half-lives of 45 days, 48 days, 87 days, and 250 days, respectively), the position is intermediate between the two extremes mentioned above.

In addition to loss of materials due to decay, care must be taken to avoid radiation decomposition when substrates of very high specific activity are used. High specific activity compounds are frequently best stored *in vacuo* at low temperatures as a thin film of dry or frozen material spread

round the walls of the containing vessel. In the case of substances supplied in dilute acid solution (many amino acids) some manufacturers recommend storage refrigerated but not frozen. The usual precautions must also be taken with appropriate substances to prevent decomposition by light, microbial contamination, etc. As radioactive substances are frequently expensive, and are often cheaper when purchased in larger amounts than would normally be used for one experiment, storage problems are more acute than with unlabelled materials.

All forms of ionizing radiation present a hazard to health; the various types of radiation emitted by radioactive isotopes are no exception, and care must be taken to avoid deleterious effects to the investigator (who is aware of the nature of the substances he is handling), and more particularly to others who may have no knowledge of the presence of radiation. Before commencing work with isotopes reference should be made to a handbook of health precautions.[1,2] In general it is as well to treat these substances as if they were powerful toxins (which indeed they are): ingestion through the mouth, nose and skin must be avoided, waste disposed of in a harmless manner, and room contamination through accident and spillage promptly dealt with before unsuspecting persons can be affected. It is useful to have available as a monitor a ratemeter equipped with a loudspeaker and a counter tube suitable for the isotope being used, so that contaminated areas may be quickly located and isolated, and contamination on the skin of the operator removed by washing and then checked with the monitor to ensure complete removal. Isotopes which emit γ-rays or hard β-rays (Br^{82}, I^{131}, Fe^{59}, Hg^{203}, P^{32} and Zn^{65}) should be confined to suitably shielded containers, both to prevent health hazards to workers and to prevent spurious results with adjacent counter tubes or the fogging of photographic film. The experimenter must take account of the release of volatile material in the course of

manipulation. For instance, when labelled bicarbonate or acetate are used as the substrates in biological systems, and chromatograms are subsequently run in an acid solvent, active carbon dioxide and acetate may be released into the atmosphere. If labelled aminoacids are treated with ninhydrin some release of carbon dioxide from the carboxyl groups occurs.

Solutions of radioactive materials should never be pipetted by mouth. For the small volumes of solution used in chromatography, micro-pipettes are available which obviate suction by mouth. Those of the Lang-Levy constriction type are accurately calibrated to hold or deliver various small volumes of liquid and are filled and emptied by means of a rubber tube and finger pressure. However, when more than very small volumes of liquid are to be applied to the chromatogram, such that the pipette may be allowed to drain entirely at one time, these pipettes are not entirely satisfactory, as slow controlled outflow of liquid from them is difficult. In practice, syringe-controlled untramicro transfer pipettes calibrated to one mark, and available in a range of volumes from 1 or 2 µl. to 500 µl., give more satisfactory results. These pipettes are filled by withdrawing the plunger from a modified tuberculin syringe, and liquid is expressed by gentle inward pressure on the plunger. All sizes of pipette fit into the same syringe. The pipettes are made with long, slender tips and possess a safety bulb, and are admirably suited to chromatography. Syringes can be made by fitting a short length of thick-walled plastic tubing over the Luer fitting on a standard 1 ml. tuberculin syringe. When larger volumes are to be applied to chromatogram origins, the syringe may be held in a clamp, with the point resting on the origin of the chromatogram, and small volumes of liquid periodically expressed onto the paper. In this case, and in many others, and where compressed air (preferably filtered) is available as a laboratory service, a

small spotting frame for holding the filter paper in the correct position may be constructed, air being blown simultaneously onto both sides of the paper, while liquid is run in from the pipette. Such an apparatus is shown in Fig. 1. Further refinements consist of an automatic drive to depress the plunger of the syringe at a rate which is just sufficient to cause the compressed air to dry the liquid into an origin spot of permitted size.

LOCATION OF RADIOACTIVE SUBSTANCES ON CHROMATOGRAMS

One of the simplest ways of finding the position of radioactive spots on one-dimensional chromatograms is to cut the paper into sections, elute the material from each section, dry the elute onto planchettes, and count the activity on each planchette in the conventional manner with an end-window Geiger-Müller tube in a lead castle. Alternatively the eluate may be counted in a scintillation counter using a scintillator solution which will accept a reasonable amount of water.[4] If a sufficiently large castle is available, it may be possible to place the pieces of paper themselves into the castle and count them without elution. However, the first two of these methods involves a prohibitive amount of work, with possible inaccuracies if the substances cannot be quantitatively eluted from the papers, and usually with a loss of the substances themselves for further study once they have been dried onto planchettes or mixed with scintillation solutions. Errors may also arise as a result of mixing of substances eluted from closely adjacent spots. The third method suffers from poor sensitivity, the possibility of cutting spots in half, or of finding two spots on the same piece of paper, and in any case both methods can be used only with one-dimensional chromatograms. Neither is recommended if any alternative is available.

Location by End-window Geiger-Müller Tubes. A chromatogram may be scanned with a Geiger-Müller tube moved manually along the paper, which is thus counted section by section. The tube is connected to a ratemeter or a scaler, and the position of active spots found. Adjacent areas to the one being scanned at any one time may be shielded with a suitable mask. This method is applicable mainly to one-dimensional chromatograms, and is discussed further in the section dealing with the Scott tube (p.).

Radioautography. The most satisfactory method for spot location on two-dimensional chromatograms, and which can also be used for one-dimensional papers, is to place the chromatogram in contact with X-ray film for a suitable length of time, and then develop the film: areas of blackening on the film will correspond with active areas on the paper.

Location with Crossed-Wire Spark Chambers. This technique is discussed in detail in Chapter and will not be considered further here.

Location by Automatic Scanning Devices. One-dimensional chromatograms may be passed through or in front of a counting tube and an automatic plot of the position and activity of radioactive areas obtained by connecting the counting tube through a ratemeter to a pen-recorder. Automatic scanning equipment for two-dimensional chromatograms is very much more difficult to design and no really satisfactory ones have been developed.

Each of these techniques has its uses and will be described separately.

Automatic Chromatogram Scanning

One-dimensional chromatograms or electrophoretograms are ideally scanned for radioactivity by an automatic mechanical apparatus. Several instruments for this purpose are available commercially. In the simpler versions the chromatogram strip (up to about 2 ft. long) is mounted on a drum which is

enclosed in a gas-tight housing. The drum is rotated in its housing thus moving successive areas of the chromatogram past a slit in the outer wall of the drum housing. A gas-flow detector tube is mounted on the far side of the slit and receives radiation emitted from that part of the chromatogram opposite the slit. The pulses received by the detector tube are fed into a ratemeter the output of which is connected to a pen-recorder. The quantitative linear distribution of radioactivity on the chromatogram strip is thus reproduced on the chart. The more elaborate instruments feed the chromatogram strip from one spool to another along a narrow slot in the sides of which are two slits exactly opposite one another. A detector tube is arranged on the far side of each slit, facing the chromatogram strip and one another, and the total output from the two tubes is fed into a ratemeter and pen-recorder as before. This instrument has two advantages over the simpler type: a much greater length of chromatogram strip may be accomodated (up to 50 ft.) and the radiation emitted is counted simultaneously from both sides of the paper. Not only does this increase the total number of counts recorded per unit time, but it avoids the problem of different intensities of radiation being emitted from the two sides of the chromatogram.

Such instruments employ window-less counting tubes for maximum sensitivity; such tubes must be flushed continuously with a special gas mixture. The resolution of these instruments is determined by the slit width employed together with scanning speed. The width of the slit determines the distance along the chromatogram which is being scanned at any one time; thus, the narrower the slit the higher the resolution, and a narrow slit should be employed with a slow scanning speed or areas of low activity may be missed if too brief a period is spent in front of a very narrow slit. The commercial instruments usually provide several slits and a variety of scanning speeds.

Irrespective of the slit and drive speed employed it will be necessary to obtain one or more correspondence points between the chromatogram and the chart. The chart trace is either marked by hand to correspond with a well-defined point on the chromatogram, such as the origin or front, or is marked automatically as a radioactive ink line passes through the counting chamber.

On laying the chromatogram and chart trace side by side with their correspondence points in the correct positions, the location of the radioactive bands on the paper is found. Other landmarks on the chromatogram (pigments, U.V. absorption, fluorescence, etc.) may also be noted on the chart as a record of the experiment. The areas under the peaks on the chart are proportional to the quantities of radioactivity on the chromatogram. The instrument may be calibrated by including on a scanning run a strip of paper bearing one or more spots of known radioactivity. The counting efficiency of window-less scanners is about 1-2% of tritium and 5-10% for C^{14}.

Two-dimension chromatograms present an entirely different problem.[5] 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 is the resolution, but the lower the number of events detected per unit time. To achieve sufficient resolution to distinguish between adjacent compounds on complex chromatograms of the type routinely obtained in some metabolic studies (see following Chapter), 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.

As a result of these difficulties no satisfactory instrument has yet been constructed which will simultaneously scan and quantitatively evaluate a two-dimensional chromatogram. However, it is possible to measure automatically the radioactivity on spots from a two-dimensional chromatogram once these have been accurately located by radioautography.[5] The spots are cut out from the chromatogram and are sandwiched at suitable intervals between two continuous bands of thin plastic polyester film wound up onto a spool. The double layer of plastic film bearing the chromatogram spots is then unwound automatically and 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. Although location of the radioactive spots on the chromatogram must still be obtained by radioautography, the automation of the counting itself permits the design of experiments involving hundreds of chromatograms and thousands of spots if this should be necessary. This equipment is not currently available commercially.

Radioautography: Use of X-ray Film

The location of radioactive spots with X-ray film may be used with one- or two-dimensional chromatograms, but is particularly valuable with the latter, as no matter how a two-dimensional chromatogram is cut into strips for scanning, there alwyas remains the possibility of overlap of two adjacent spots if these lie diagonally in relation to the direction in which the strips have been cut.

Type of Film. The speed of the film and its sensitivity to the radiation emitted by the isotope are the main considerations in selection of a suitable film, the grain being of little account. No-screen X-ray films as made by the usual manufacturers are the most suitable for radioautograms of chromatograms, and as their name implies, these are designed for use without intensifying screens. For weak emitters (C^{14}, H^3 and S^{35}) single coated films are to be preferred, as they should be cheaper, the energy of the particles is too low for them to penetrate the film base and affect the emulsion on the side away from the paper chromatograms, the presence of only one emulsion reduces the background (from cosmic rays, etc.) to one-half of that with a double-coated film, and there is a saving of developer and fixer. Other advantages of single-coated film will be mentioned below. A suitable film is Kodak Single Coated Medical X-ray Film (Blue Sensitive) from Eastman Kodak Company, Rochester, New York, U.S.A.; when single-coated X-ray film is not available, double-coated film can be used. This is marketed by Kodak Ltd. as "Kodirex," and by Ilford Ltd. as "Ilfex."

Storage of Film. Film should be stored in cool, dry conditions, and should be used before the expiration date indicated on the package. Particular care must be taken to avoid storing film where it can be fogged by radio-isotopes, X-ray machines, etc. When chromatograms have to be exposed

to film for prolonged periods it is best to store the film during exposure in an underground cellar where it will be less subject to fogging by cosmic radiation: for weak β-emitters, single-coated film is also desirable because of the reduced background (v. supra).

Exposure Period. The length of time necessary to expose a film to a chromatogram of radioactive materials depends on the amount of activity per unit area on the paper, the nature and energy of the radiation emitted, and the half-life of the isotope. With C^{14}, for example, a spot about 1 cm.^{2} in area emitting about 5,000 particles per minute from the surface on each side of the paper will produce a barely detectable blackening on the film in one day. One should aim for at least ten times this activity, or ten times the exposure period. With tritium the time of exposure would be greater than with C^{14}, due to the very low penetrating power, while with P^{32} both emulsions on a double-coated film are affected, and the exposure time is reduced. A chromatogram may contain some spots very much weaker than others, and the exposure time must be chosen accordingly, as a film exposed for the more active compounds may fail to show any signs of the weakly active components. However, in this case, over-exposure of the strongly active spots has no deleterious effects on the film as a whole. Lastly, there is no useful purpose served by exposing a chromatogram to film for more than three or four times the half-life of the isotope. Exposure for the third half-life contributes an extra exposure factor of 16.7 per cent of the accumulated exposure for the first two half-lives; the fourth half-life contributes an extra 6.7 per cent of the accumulated first three half-life exposures, while the fifth half-life gives only 3.2 per cent more than the exposure already achieved.

Radiochemical Contamination. It may be necessary to ensure that the active material used (substrate) contains no significant amount of radiochemical contamination; a significant amount may be quite small. For instance, if a substrate contains 1 per cent of a contaminant, and 10 per cent of the substrate is used in a reaction to produce ten equally active products, then each product in the mixture will have the same total activity as the original contaminant, assuming the latter plays no part in the reaction. The contaminant may cause confusion by being incorrectly interpreted as a product of the reaction, or interference may result by the contaminant chromatographing together with a product. In such cases preliminary purification of the substrate by chromatography in the system to be used later for analysis is advisable. After elution, the substrate should be used soon if there is any evidence that the contaminant may be reformed from the substrate, perhaps by radiation decomposition.

Labelling of Film. For alignment of the radioautogram with the chromatogram after development, it is advisable to stamp or number the chromatogram in two places (away from areas likely to contain spots) with radioactive ink. The stamp may be cut from a rubber stopper and an asymmetric design is preferable so that no confusion can result between the two stamps on each paper. The ink mark should, of course, be made after the chromatogram has been developed in solvent and dried. A suitable ink is made by mixing an appropriate quantity of radioactive material (a long-lived isotope is best, to avoid frequent preparation of ink) with black endorsing ink. Enough radioactivity should be used to achieve an adequate exposure in the time that the film will be lying next to the paper. A simple stamp pad can be made from several layers of gauze cut to shape and pressed into the bottom of a flat-bottomed tube, and enough ink added to give a moist pad. The rubber stopper is mounted on a glass rod bearing a cork at the other end as a handle.

Films can be labelled by writing on them with a hard pencil point on the emulsion side before development, preferably at the time the film is placed against the paper, so as to avoid confusion. The pressure of the pencil point is sufficient to form a latent image on the film. If this is forgotten at the time, films can also be labelled after processing by writing on them in ink, but this results in a less permanent image.

Exposure Techniques. The manufacturers supply X-ray film in a variety of sizes and packings. Owing to its high cost, wastage should be avoided, the nearest standard size available for the 10 in. (25.4 cm.) square papers described in this book usually being 10 in. X 12 in. (25.4 cm. X 30.5 cm.). Films are boxed envelope-wrapped and folder-wrapped. In the latter case each film is inserted in a simple paper folder which does not constitute a light-tight enclosure. Films in this packing are cheaper and are satisfactory, unless it is desired to use the envelope from envelope-wrapped film as a container for the film during exposure to the chromatogram, but folder-wrapped film may be taken out of the packet only under recommended dark-room conditions. Envelope-wrapped film is first folder-wrapped and then enclosed in a light-tight envelope together with a piece of stout card.

During the exposure period the film may be kept in one of several containers. Individual film-chromatogram pairs may be stored in envelopes as mentioned above, or in the special exposure holders supplied by photographic manufacturers, which can be used repeatedly, and do not require envelope-wrapped film. They are supplied in sizes appropriate for the various sizes of film. When large numbers of chromatograms are to be exposed to film all for the same period, the film-chromatogram pairs, each in its folder, can be stacked together between two sheets of card (supplied in boxes of film) and then stored in the light-tight boxes in which folder-wrapped film is sold. The lids of the boxes should be kept sealed with adhesive tape during exposure to prevent accidental opening.

If such a size of paper and film is being used that it becomes necessary to wrap the edge of the paper round the film, there is a possibility with double-coated film of obtaining an image on the back of the film due to the wrapped-over edge of the paper. In addition the back emulsion of one film may be exposed to particles from the back of the next paper, penetrating two layers of folder paper even with weak emitters like C^{14} and S^{35}. In such cases it is best to place an old sheet of developed film between each film chromatogram pair; if the edges of the paper are to be wrapped round the film, then they can instead be wrapped backwards round the intervening sheets of old film.

Precautions must be taken with γ-emitters and strong β-emitters which are unnecessary with weak β-emitters such as C^{14}, H^{3}, and S^{35}. If chromatograms bearing P^{32} and Cl^{36} are to be stored in stacks of envelopes or in boxes, sheets of absorbent material (3 mm. aluminium sheeting) must be interleaved between each chromatogram to prevent exposure of the film by particles emitted from adjoining papers and penetrating the folders, envelopes and chromatograms. The special exposure holders supplied by the manufacturers are backed with lead foil and this should be adequate in preventing such cross-contamination. Chromatograms of γ-emitters must not be stored in stacks unless thick lead screens can be provided between adjacent films, but must be stored flat all on the same plane, when cross-contamination will be minimal and can, in effect, be ignored. Table 1 summarizes a number of properties and more important practical details of isotopes most commonly used in biological research.

Although adequate contact between film and paper may be obtained by packing in an envelope or fairly full box many workers use weights to maintain a good contact. If these are used with film kept in thin packages (such as envelopes) it should be remembered that weights with sharp edges are

liable to cause pressure markings on the films in the same way that writing on them with pencil will do, and diffuse weights covering the whole area of the film are preferable (e.g. sand bags, or large sheets of metal). Films being exposed, like fresh film, must be stored away from external radiation from γ-emitters and X-ray machines.

Processing Films. For loading and processing X-ray films dark-room facilities are necessary. Safelights may be used according to the processing instructions which are supplied by the film manufacturers on request. During processing, the films can be processed in dishes of developer, washing water, and fixer, but if films are to be processed routinely a deep processing unit is most convenient. These units are supplied either in one piece to be connected with the plumbing in the building, or as sets of separate tanks, the latter, of course, being much less expensive. The films for processing are mounted in special frames. With channel hangers the film slides into grooves which hold it on four sides, but quite frequently falls out during processing. Tension hangers are much to be preferred as the film is impaled at each corner and then held flat and rigid under tension. With both types of hanger the films are placed vertically into the processing baths, and several films may be processed simultaneously.

Viewing and Intensification. After processing and washing, films are dried by allowing them to stand vertically overnight (still in their frames, or hung from a line with a clip), and drying may be accelerated with a current of air, not above 40-45°C or shrinkage of the film will result. Films may be viewed against a white background or against the window of a viewing box: the best viewing boxes have fluorescent lights and a dimmer switch. Very faint spots on the film are best seen with a dim light in the viewing box, and can often be seen more easily if the film is moved rapidly

back and forth through a small distance than when stationary. If for some reason only very faint spots can be obtained on the film, the image can be intensified by blue toning. This is normally done immediately after the film has been washed in running water after fixing, but old films can also be intensified after soaking them in water for half an hour in order to soften the emulsion. The directions are as follows:[6]

Solution A	Water (about 20°C)	750 ml.
	Diglycollic acid (α, α'-oxydiacetic acid)	60 g.
	Sodium hydroxide	36 g.
Solution B	Water (about 20°C)	100 ml.
	Ferric nitrate. 9 H_2O	14 g.
	Potassium fluoride. 2 H_2O	1 g.
Solution C	Water (about 20°C)	100 ml.
	Potassium ferricyanide	5 g.
	Sodium nitrite	1 g.
Total volume		1000 ml.

Solutions A, B, and C are prepared by adding the constituents in the order given above. Solution B is added to solution A in 2 min. with stirring. Solution C is added to solutions A plus B in 2 min. with stirring. Stir 1 min. The sodium hydroxide and diglycollic acid act as a buffer and the final pH should be about 5. Potassium fluoride acts as an antifogging agent, and sodium nitrite as an anticorrosion agent. The solution is reported to be stable in stoppered amber-coloured bottles for at least six months.

The film is totally immersed in the intensifying solution at 20°C for 2-8 min. depending on the degree of intensification required. It may be removed during this period for inspection; the process is carried out in normal room lighting. The films are washed in running water for not more

than 5 min. and dried in the usual way. The blackened areas on the film are turned deep blue and if the resultant image is viewed with red light, or through a red filter, an increase in film speed of about five times can be obtained; i.e., the same result is produced after intensification as would require an exposure five times as long with no intensification. This technique is especially valuable when very long exposures would otherwise be necessary, or when further exposure is pointless due to the short half-life of the isotope.

Having located the spots on the film with the viewing box, they are ringed round with wax pencil, which can easily be removed if necessary by rubbing with a paper tissue or soft cloth. Ink should not be used as it is very difficult to remove satisfactorily. The outline of the spot is then traced from the film to the paper after the two have been lined up using the ink marks. Films tend to shrink after prolonged storage, and should the occasion arise, it may be necessary to expose the paper again after a long period if the location of a spot is required with great accuracy. Developed films with their chromatograms are conveniently stored in the folders in stacks. The wrappers may be labelled, and it is highly desirable that each film should bear at least the same reference number actually developed into the emulsion, as appears on its corresponding paper.

Radioautography of Tritium-containing Chromatograms. Although chromatogram spots containing tritium will produce satisfactory radioautograms if sufficient activity is present,[7] a technique has been proposed[8] which can greatly shorten the exposure time necessary for tritium chromatograms. After development in the solvents, the chromatograms are dried and attached by staples in the corners to a suitable backing material (e.g. used X-ray film). The backing sheet and chromatogram are laid with the latter uppermost in a shallow tray and covered with scintillating liquid (e.g. 3 g./l.

of p-diphenyl benzene in sulphur-free toluene). A sheet of film is laid in the liquid on top of the film, taking care that no air bubbles are trapped. They tray is then covered with a light-tight and air-tight lid, and the film left for as long as necessary. The film is removed from the tray, dried, and developed and fixed in the usual way. This technique can, of course, only be used if the tritium-containing substances are insoluble in toluene.

Spot Identification. The chemical identity of radioactive substances on chromatograms is inferred in the first place from the chromatographic position with the solvent systems employed. To confirm identity, and assuming that the amount of material on the paper, while sufficient to cause film blackening, is not enough to give a chemical colour test, the substance is eluted from the paper, and co-chromatographed with authentic marker substances in sufficient quantity to give a chemical reaction. The chromatogram of unknown active compound plus unlabelled marker is again exposed to film, and after development of the film and the colour test on the paper, the two are accurately placed together using the ink stamp for correct alignment. If the unknown is identical with the marker, the film blackening and coloured spot on the paper should correspond in every detail: position, shape, size, details of marginal irregularities, etc; this is known as "fingerprinting." It may be desirable to repeat co-chromatography in more than one solvent system.

Chemical treatment of active substances may be followed by chromatography of the products in the usual way, except that changes will be looked for among radioactive substances, instead of among compounds detected by colour tests. This can be of the greatest value in the isolation of minute quantities of substances from biological systems, in amounts suitable only

for ultramicro manipulation. Substances giving only a few hundred counts per minute can be subject to many different chemical reactions and the results analysed chromatographically if one is prepared to wait long enough for a film image to be formed. Further reference to this aspect of the technique will be made in the following chapter (p.).

Specific Radioactivity. Finally, it must be borne in mind that a suitable specific activity of labelled compounds on chromatograms is necessary. Only a limited quantity of material in terms of weight can be adsorbed by a sheet of chromatogram paper, and if too little labelled material is used in a system which already contains large amounts of unlabelled substances, the radioactivity may be too dilute for the film image to be formed in a reasonable time. Further, if quantitative measurements are to be made by assaying the radioactivity in the spots, it is desirable to have reasonable activities, or counting may be prolonged very greatly, and the counts subject to large errors.

ASSAY OF RADIOACTIVE MATERIALS IN CHROMATOGRAPHY

After the position of the radioactive spots on the chromatogram has been found, the operator has the choice of determining the amount of activity in each spot either by eluting it and counting in a suitable system, or by counting directly on the paper. Elution followed by counting has advantages, with weak β-emitters, of an increased efficiency compared with counting directly on the paper, and this is of importance when very low activities are being employed.

In many cases, assaying the spots for radioactivity directly on the paper can be performed, using the Scott-type Geiger-Müller tube described

below. Time is saved by doing this, but some sensitivity is lost with weak β-emitters due to absorption of radiation in the paper. However, for most types of experiment it is the most economic way of determining spot activity, and is non-destructive of the compound under study. Automatic counting devices have been discussed above (p.).

Half-life and Counting

The length of the half-life of the isotope can be of importance in counting. With isotopes of long half-life (C^{14}, Cl^{36} and H^{3}) a spot counted twice at, say, an interval of one month, should show no difference in activity, but with short lived isotopes there might be considerable diminution of activity after this period. Thus a spot of P^{32} would give only 25 per cent of its original activity after four weeks, I^{131} only about 6 per cent while Br^{82} would give only 0.0002 per cent. A simple way to avoid complex calculations of the amount of decay which has occurred up to the moment of counting is to prepare a standard planchette or spot on filter paper of the isotope being used at the beginning of the period when counting is to take place. The standard is then counted periodically and the factor necessary to convert the observed count at any particular period to the count when the standard was first prepared is calculated. Thus experimental readings made at a certain time are multiplied by the same factor as that necessary to convert the standard sample to the original value. A correction of this sort is essential if the counting operations for one set of results occupy a significant fraction of the half-life of the isotope.

Scott-type Geiger-Müller Tube

The end-window tubes supplied by manufacturers usually have a window of mica with a thickness density of 2 mg./cm.^{2} or more. When weak β-emitting

isotopes are to be employed, and these are important in biochemical research (C^{14}, H^3, and S^{35}), the attenuation of radiation by the window can represent a considerable proportion of the total. Thus, at a window thickness of 2 mg./cm.^{2}, no β-particles from tritium would pass into the sensitive volume of the counter tube, and with C^{14} some 25 per cent would be absorbed in the window material. In addition, mica windows are very fragile, the counter tubes are expensive, and have a limited life. For these reasons the type of Geiger-Müller tube to be described here has many advantages for chromatographic use; the tube was originally designed in the Lawrence Radiation Laboratory of the University of California, and a constructional diagram has been published by Fuller[9] and is reproduced in Fig. 2. A photograph is shown in Fig. 3.

Construction. The tube is constructed from brass stock tubing, 0.25 in. (0.64 cm.) thick, and 2.5 in. (6.35 cm.) external diameter. The inner surface of the tubing is machined and polished to an internal diameter of 2.25 in. (5.72 cm.). A flange is fitted at the bottom, open, end over which the window of thin plastic film (see below) is fitted and held in position with a stout rubber O-ring. Copper tubes are provided for gas flow, and the top of the tube has a hole into which the central tungsten wire assembly is fitted. The top may be constructed of brass, but unless tritium is to be the isotope counted, it is often more convenient to have the top constructed of Perspex so that the exact location of the counter tube on the chromatogram can be observed. In this case, however, it is necessary to fit an earth connection between the outer brass tube and the casing of the central high voltage wire assembly. All interior metal surfaces of the tube should be highly polished, and all sharp edges and corners, such as the entry ports for the gas tubes, and the bottom edge, must be well rounded

off to prevent discharge from the central high tension wire. The point of the tungsten anode is insulated by a small glass bead fused onto the end. A flexible cable connects the tube with a conventional scaling unit: this cable should be as flexible as possible to permit the placing of the counter tube at any position on the chromatogram.

Window. The window material consists of thin plastic polyester film, sold in the United Kingdom under the trade name of "Melinex O" (I.C.I. Sales Office, 81/87, High Holborn, London, W.C.1.), and in the United States as "Mylar" (E. I. DuPont de Nemours & Co., Inc., Wilmington 98, Delaware), and is supplied in rolls of several hundred yards of plastic film of varying widths and thicknesses. For use with the Scott tube, 6 in. (15.25 cm.) is a convenient width and the thickness required is 25 gauge (0.00025 in., 0.00625 mm.), having a density thickness of 0.86 mg./cm.^{2} To fit the window, a 6 in. (15.25 cm.) length of plastic film is cut from the roll, fitted roughly over the end of the tube, and held in place by the rubber O-ring stretched over its flange. The plastic is then pulled taught by gentle tension at various points round the tube, and care must be taken to avoid wrinkles in the film, and particularly tear holes: these are especially likely to occur where the plastic passes over the flange, usually when too great a tension is used when pulling the window taught. At any time the window may be replaced in a few seconds should it become damaged or contaminated.

Operation.

Gas Filling. The counter tube is designed to be operated as a gas-flow counter for two main reasons: in the first place, the gas filling of the tube needs to be replaced each time the window is removed, and secondly the thin plastic film itself is slightly porous and gas must continuously be

passed through the tube to prevent the entry of air. Two types of gas filling are possible, "proportional gas" and "Geiger gas." The former consists of a mixture of 90 per cent argon and 10 per cent methane, and has the advantage of being relatively cheap, and the disadvantage of requiring a very good high voltage supply for use in the proportional region, and has an operating voltage of about 2.4 kV. Many scalers are provided with high voltage power packs giving only 2 kV. Of the Geiger gases, "Q" gas is the one of choice (99.05 per cent helium and 0.95 per cent iso-butane), but is extremely expensive in Europe due to transportation across the Atlantic Ocean in cylinders.

In practice the most convenient gas to use is helium (American Mineral Grade) bubbled through ice-cold ethanol to provide a quenching agent (bubbling tube 2.5 cm. diam., ethanol about 10 cm. deep, gas inlet tube 0.14 cm. int. diam.). As the counter is not intended to be run for long periods in the absence of an operator, it is not inconvenient to maintain a bubbling tube containing ethanol in an ice bath in a wide neck vacuum flask. One or two fillings with ice is usually sufficient to cover a full day's operation. The gas should flow through the counter tube at the rate of about 5 bubbles per second, and a long rubber tube fitted to the outlet port prevents the diffusion of air back into the counter tube. Before use each day the tube should be well flushed with the helium-ethanol mixture (increasing the flow rate to save time) and then the tube is completely filled with counting gas and the air expelled, the gas flow rate can be adjusted to 5 bubbles per second. The gas flow rate is not critical and the operator should experiment with his own tube to achieve a flow rate sufficient to prevent air entry, but not too wasteful of the helium. Failure of the tube to function properly is frequently due to the gas flow being too low. In the event of the counter not working well the first things to check are that the gas flow is

adequate and that all electrical connections are tight. The high voltage should be switched off whenever the window is changed or electrical connections dismantled.

Plateau. The Scott tube has an operating plateau[1,2,3] which varies somewhat from tube to tube, and with different gas fillings. Typical values are, with "Q" gas, a plateau from 1,550-1,800 volts, and with helium-ethanol, a plateau from 1,750-1,950 volts. The plateau for each tube should be determined, and the counter operated at a voltage about 100 volts higher than the beginning of the plateau. Thus, the tube can be satisfactorily operated with a power pack delivering only 2 kV. The plateau has a slope of about 5 per cent/100 volts at the operating voltage. The counting tube is used without a shield, and the background in the absence of neighboring interference from X-ray machines, etc., is about 100 counts/min. The background count should be taken periodically during the counting operations under the same conditions as the experimental counts are made and should, of course, be subtracted from the experimental values obtained in a similar counting time.

Standard Sample. It is convenient to prepare a standard sample of some long-lived isotope with which the working of the counter tube may be routinely checked at the beginning of each working day, and after the replacement of windows, repair of faults, etc. The standard sample may be prepared from a piece of radioactive plastic sheet, or by evaporating a small volume of a solution of radioactive material onto a flat thin metal planchette, and covering the dried material with a thin layer of varnish to minimize loss from the planchette by rubbing, etc. C^{14} is a suitable isotope to use in preparing a standard planchette, but should not, of course, be contained in a volatile substance. If the absolute efficiency of the counter is

required, it is best to prepare the standard planchette with a known amount of the isotope to be used experimentally, and then to observe the counting rate with the particular tube involved. If the total activity on the planchette is known, the percentage of the total disintegrations recorded by the counting system can be calculated. For routine use, sufficient radioactive material should be placed on the planchette to give a counting rate of about 4,000 counts/min. This is well above background, and a count for 1 min. will be subjected to a coincidence error of only about 1.6 per cent.

Static Charges on Window. It is sometimes found that the plastic window acquires a high static charge, particularly when the atmosphere is very dry, and when the tube is standing on certain surfaces. This can be removed by wiping the window with a paper tissue moistened with ethanol, and allowing the window to dry. The window can also be cleaned in this way from dust, and sometimes from contamination as well. Some workers keep an open vessel of water on the boil in the counting room when the humidity is very low. It is also possible to avoid the problem of static charges by using as windown material thin plastic sheet which has been sputtered on both sides with a very thin film of gold or aluminium. This renders the window material conducting without at the same time adding any significant amount of mass or rendering it totally opaque.

Counting the Spots. Having been located by the blackening on X-ray film (or other method), and their positions traced from the film onto the chromatogram paper, the spots are counted simply by placing the Scott-tube on the paper, and observing through the Perspex top that the tube is properly alighned over the spot. With spots giving some thousands of counts per minute it may be sufficient to count only for one minute. If the counting rate is very high, coincidence corrections should be applied. For weaker spots it may be necessary to count for more than one minute, but in all

cases it is usually convenient to express the activities in terms of a standard time unit, often 1 min. or 1 sec.

Attenuation of Active Spots. When spots are to be assayed which give counts so high that they cannot conveniently be counted with the counter (over about 30,000 counts/min.), the activity reaching the counter may be attenuated by placing a shield of a material suitable for the isotope between the filter paper and the counter window. The degree of attenuation necessary will depend on the activity in the spot and the nature of the isotope, and convenient shields may be found by experiment. With C^{14}, for instance, a sheet of aluminium foil forms a suitable shield and the degree of attenuation can be determined by counting the standard planchette with and without the shield. Aluminium foil can also be used for S^{35}; with higher energy β-emitters such as Cl^{36} and P^{32}, steel or lead foil are better, as aluminium sufficient to give a several-fold attenuation is too thick for convenience. It should be noted that the background count is not altered with an attenuating shield, and background should consequently be subtracted from the observed count before the latter is multiplied by the attenuation factor.

Shielding Adjacent Spots. Spots on the chromatogram adjacent to the one being counted must be completely shielded if they are close enough to be covered, wholly or partially, by the counter window. This is most simply done by cutting the spots out of the chromatogram and counting each one individually under the tube. If it is desired to keep the whole chromatogram intact then masks are necessary to absorb the radiation from the undesired spot. Masks to shield out adjacent spots are similar to attenuating shields save that 100 per cent attenuation is required. Again, the type and thickness of shield material depends on the isotope being used, and examples of suitable masking substances are given in Table 1.

Owing to the low sensitivity of the Scott-tube for γ-rays, it is often sufficient with isotopes which emit both β- and γ-rays to shield only the β-particles from adjacent spots to achieve virtually complete masking. This is the case with I^{131}. With Br^{82} and Fe^{59} about 4 per cent and 1 per cent, respectively, of the counts in the absence of an attenuator are obtained when the chromatograms are counted through a sheet of stainless steel 1 mm. thick. If this residual counting rate is acceptable, adjacent spots may be masked with pieces of sheet steel of suitable shape. When it is important that there should be no interference at all from neighbouring spots, the spots to be counted must be cut from the chromatogram and counted separately.

With Zn^{65}, about 97.5 per cent of the decay is by K capture of electrons, with accompanying γ-emission, and about 2.5 per cent by positron emission. There are thus a very large number of γ-rays emitted for each countable β-emission, and these prevent adequate shielding even though the sensitivity of the counter for γ-rays is low. A spot of Zn^{65} on paper still gave 31 per cent and 27 per cent, respectively, of the counting rate without attenuator when counted through 1 mm. of stainless steel or 1.65 mm. of lead. Spots on chromatograms containing Zn^{65} must therefore be cut out and counted separately.

Where possible, shields of materials which can easily be cut to shape are desirable: index card in the case of C^{14} and S^{35}, and writing paper with H^3. For the counting of most of the spots on the chromatogram it is useful to cut out beforehand a number of masks of standard shapes: right-angled corners, two or three each of convex and concave curves, etc. It is often found, however, that two spots lie so close together, or have such awkward shapes, that it is difficult to deal with them unless masks specially cut

to the shape of these spots are used. The shape of such spots can be traced onto the masking material and the spots cut out. Developed X-ray films are also very good masking materials for C^{14}, S^{35} and H^{3}, and have the advantage of being transparent. Sometimes a spot, too large to be covered by the counter at one time must be counted. It then becomes necessary to subdivide the spot into sections and count these one at a time, while shielding out adjacent sections. It is particularly important in these cases to ensure that the edge of the mask lies truly on the dividing line, otherwise small areas of overlap may be counted twice; and, of course, whenever a spot is counted in sections, the background must be subtracted for each section counted. Care must also be taken when counting a very weak spot lying close to a very strong spot that the strong one is completely shielded. If it is necessary for the counter window partly to cover the shielded strong spot, it might be advisable as a safely precaution to place double the normal amount of shielding material over the stronger spot, as even a very small percentage of the activity from the strong spot passing through the shield may be equivalent to a large percentage of the activity from the weak spot. In extreme cases, where shielding might be impracticable, the weak spot can be cut out from the chromatogram and counted separately, quite safe from any possibility of interference from a neighbouring strong spot.

Counting Efficiency. With the lower energy isotopes it is desirable to count each spot on both sides of the paper, and to average the readings. Unless chromatogram papers are dried several inches apart, and this is often not convenient, solvent evaporates more rapidly from one side of the paper than from the other, leaving proportionately more labelled substance on that side. This is not important with strong emitters, but with C^{14}, S^{35},

and H^3 the radiation is weak enough to suffer serious absorption in the paper itself, resulting in a different counting rate from each side of the paper. With Whatman No. 4 paper, for instance, a standard amount of a C^{14}-containing compound gives only about 33 per cent of the counts when spotted onto filter paper compared with drying onto a flat planchette. With H^3 the corresponding value is only about 8 per cent but with a strong β-emitter (P^{32}) the value rises to about 95 per cent, and counting both sides of the paper is hardly necessary. In this connection it is worth noting that with weak β-emitters, counting the origin of the chromatogram on both sides of the paper before the chromatogram is run almost invariably gives a count higher than the sum of all the spots on the developed chromatogram. One side of the origin is liable to give a count twice as high as the other, or even higher. This appears to be due to the fact that the liquid is usually dried preferentially onto one side of the paper, resulting in faster evaporation and more deposition of active material on that side. Further, material lying at the origin appears to be lying closer to the surface of the paper than in spots on the developed chromatogram, and there is thus less absorption in the paper itself.

The efficiency of the counter with isotopes such as C^{14} and S^{35} placed on a flat planchette actually touching the window is about 20 per cent; i.e., about 20 per cent of all disintegrations are counted. This is the resultant value of the geometry of the counting system, absorption in the window material, efficiency of ion collection in the counter tube itself, etc. With these two isotopes spotted onto Whatman No. 4 filter paper the efficiency falls to about 7 per cent. With strong β-emitters, the fall in efficiency is much less when spotted onto paper rather than onto a planchette, and workers interested in finding the ratio between counts on paper

versus counts on a planchette are advised to make their own determination for the particular isotope and paper being used. Different filter papers have varying thicknesses and densities, and hence varying powers of absorption, while various isotopes have emissions of different penetrating powers.

Reproducibility. The degree of reproducibility of counts taken with the Scott tube may be illustrated by the following example. A spot on a chromatogram containing C^{14} was counted for 12 one-minute periods; the counter was not moved between counts. The rate of counting was 3,943 ± 75 counts/min. The same spot was counted for one 12-minute period, resulting in a count rate of 3,904 counts/min. The spot was next counted for 12 one-minute periods, the counter being removed from the paper after each count and replaced as closely as possible at exactly the same position on the paper: the count rate was 3,940 ± 56 counts/min. For these counts the background was not deducted. Twelve one-minute counts of the background gave a rate of 99 ± 10 counts/min., while one 12-minute background count gave 101 counts/min. The reproducibility both of the electrical system and of the placing of the counter on the paper, using a spot of moderate activity, was better than ± 2 per cent. The error was much higher with the low rate of background counting, and this would apply equally to weak spots, counting at about the same rate as the background.

Scanning One-dimensional Chromatograms. In addition to its use for counting spots on two-dimensional chromatograms, the Scott tube may also be used as a manual auxiliary to the automatic one-dimensional scanner described in this chapter. Using a mask suitable for the isotope, and containing a slit, one-dimensional chromatograms may be scanned by hand, section by section, in order to locate the positions of active areas, if an automatic scanner is not available (p.). Location of spots in this way is more

rapid, but less precise, than with X-ray film, and has the advantage that the spots are counted at the same time as they are located.

Counting Tritium. With the exception of tritium, all the isotopes mentioned above may be assayed with the Scott tube as previously described. Tritium remains the one exception, as the energy of the β-radiation from this nuclide is so low, that the particles are completely absorbed even by the very thin window employed. However, tritium may be counted if the window is removed, and the tube used as a window-less counter. The tube, minus window, is placed on the chromatogram in the usual way, and time is allowed to elapse until all the air which has entered the counting tube after each change of position, is swept out by the gas mixture: this takes about 5 min., depending on the rate of gas flow. The isotope is then counted in the usual way, and the counter moved to the next position. Time is saved by sliding the counter along the paper from one spot to the next, so that less air leaks into the counting tube than if the counter is bodily raised from the paper and set down again. When used without a window the reproducibility is not quite so good, and the gas rate must be carefully adjusted to about 5 bubbles/sec.; the rate varies somewhat from tube to tube, and operators should find the optimum rate for their own particular equipment. The windowless tube is also rather sensitive to light, and if a Perspex top is fitted, this should be blacked out when the tube is used without a window. Alternatively, a separate brass top should be available for fitting in the event of tritium counting, or completely separate tubes may be maintained. There is no practical advantage to be gained in using a windowless tube for assaying nuclides other than H^3. Even with the next weakest isotope (C^{14}), the energy of the radiation is high enough to result in absorption by the window of not more than about 10 per cent. The extra experimental difficulty of

manipulating the counter without the window, and the time spent waiting for air to be flushed out, is not worth the gain of an extra 10 per cent or so in rate of counting. With tritium there is no alternative, unless the compound is eluted from the paper, or a liquid scintillation counter is available (see below), and the use of a windowless tube is necessary in order to obtain any results at all direct from the paper.

The Scott tube is thus a cheap, robust, and versatile instrument to use for assaying radioactivity on paper chromatograms, and renders unnecessary the prohibitive amount of time required to elute each spot from many chromatograms for counting in some other system. The reproducibility is quite adequate for most types of biological experiment, and the tube can be employed for most, if not all, of the radioactive isotopes used in biochemical research.

Liquids Scintillation Counting of Excised Chromatogram Spots. Pieces of chromatogram paper bearing radioactive material may be counted without elution by being placed in scintillation solution with or without prior treatment with solubilizer.[10,11,12] This permits automatic counting of excised chromatogram spots without the construction of the complex equipment discussed above (p.). After location of the compounds in the usual way with radioautography, the spot is cut out of the chromatogram, placed in a liquid scintillation vial and counted as described by the authors in references 10, 11 or 12. Counting in this way has the advantage of high counting efficiency as well as the ability to measure two isotopes simultaneously, and use may also be made of an automatic sample changer if this is available with the counter. The disadvantage is that it may be difficult or impossible to recover the active material for further study once it has been prepared for scintillation counting.

Thin Layer Chromatography of Radioactive Substances. Some of the techniques used for paper chromatography are also applicable to thin layer chromatograms. Radioautograms may be made by applying X-ray film, emulsion side down, directly to the chromatogram. Should any difficulty be encountered with particles of adsorbent adhering to the film, a layer of thin plastic sheet (Mylar/Melinex) may be interposed between the adsorbent and the film.

Counting of labelled compounds separated on thin layer chromatograms is usually accomplished by carefully scraping off the appropriate part of the chromatogram into a scintillation vial and shaking with scintillator solution,[13] containing dioxane and toluene[4] for water-soluble materials or conventional toluene-based solution for lipids. The particles of adsorbent are maintained in suspension by the addition of 4% Cab-O-Sil Thixotropic Gel Powder (Packard Instrument Co., Inc., Downers Grove, Illinois).[13] This technique may be used both for C^{14} and for H^3; in the latter case it is necessary to allow the sample to equilibrate for about 30 minutes with the gelled scintillation solution before counting. Scanning devices, analogous to those for one-dimensional paper chromatograms, are also commercially available for thin layer chromatograms.

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TABLE 1

Physical Properties of Several Radioisotopes and Suggestions for Radioautography and Counting with a Scott-type Geiger-Müller Tube

Isotope	Half-life	β-energy (MeV)	γ-energy (MeV)	Shielding for Adjacent X-ray Films in Stack	Maximum useful Exposure Period (days)	Shielding for Adjacent spots when Counting	Residual Count Rate when Shielded with 1 mm. Steel (%)
Br^{82} . .	36 hr.	0·465	0·55–1·3	None convenient*	4·5–7	1 mm. steel	4
C^{14} . .	5,600 y.	0·155	—	X-ray film	—	Index card	0
Cl^{36} . .	440,000 y.	0·713	—	1·6 mm. Al	—	1 mm. steel	0
H^{3} . .	12 y.	0·0180	—	X-ray film	—	Paper	0
I^{131} . .	8 d.	0·33, 0·60, 0·15, 0·81	0·08–0·72	None convenient*	24–32	1 mm. steel	0
Fe^{59} . .	45 d.	0·26, 0·46	1·3, 1·1	None convenient*	135–180	1 mm. steel	1·2
Hg^{203} .	48 d.	0·205	0·286	None convenient*	144–192	1 mm. steel	
P^{32} . .	14 d.	1·712	—	3·2 mm. Al	42–56	1 mm. steel	0
S^{35} . .	87 d.	0·166	—	X-ray film	—	Index card	0
Zn^{65} . .	250 d.	0·32	1·11	None convenient*	—	None convenient†	31

All the isotopes listed here decay to inactive daughter products and hence cause no interference on chromatograms.

* See text page † See text page

Captions for Figures

Fig. 1. Spotting frame for chromatograms. Compressed air is blown simultaneously over both surfaces of the paper. Air to the upper surface can be turned off by a tap while liquid is actually being run onto the paper. The air may be heated by placing a bunsen burner under the coiled pipe. A 100 ul. ultramicro transfer pipette attached to a syringe is also shown.

Fig. 2. Constructional diagram for the Scott-type Geiger-Müller tube.[4] All measurements are in inches (1 in. = 2.54 cm.). This figure is reporduced by courtesy of the Editor of Science.

Fig. 3. Experimental arrangement for assaying radioactivity in chromatogram spots. From left to right: illuminated viewing box for examining radioautograms; vacuum jar containing iced water and bubbling tube with ethanol; scaler, with Scott-type Geiger-Müller tube in front; cylinder of helium.

Figures

Fig. 1: Use the same block as for Fig. 25.1, page 486 of 2nd edition.

Fig. 2: Use the same block as for Fig. 25.4, page 498 of 2nd edition.

Fig. 3: Use the same block as for Fig. 25.5, page 499 of 2nd edition.

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