EXPERIMENTS ON THE APPLICATION OF AUTORADIOGRAPHIC TECHNIQUES TO THE STUDY OF PROBLEMS IN PLANT PHYSIOLOGY

By R. THAINE* and MADELINE C. WALTERS†

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Summary

A method of autoradiography has been developed which allows the record of single β-particles emitted from sections of plant tissue 5-7 μ thick. The method allows resolution of the order of 1 μ and magnification up to $\times$ 1500. Dissolution of the water and alcohol soluble contents of the tissue has been overcome, and it is therefore possible to determine the distribution of compounds concerned in the metabolism and biosynthesis of a cell. Cellular distortion and loss of contents during fixation and photographic processing is illustrated, and it is hoped that an improved method of freeze-drying will reduce this limitation. In macroscopic autoradiography the soya bean has proved to be most valuable plant material, because the thin stems and leaves allow autographs which give a detailed record of the transported compounds containing ^{14}C. The isotope is introduced to the plant in the photosynthesis of $^{14}CO_2$, and after a suitable period, the plant is dissected, dried, and placed against X-ray film. After 14 days exposure the film is developed, and the photographic image records positions and relative concentration of the isotope in the plant tissue.

I. INTRODUCTION

The earliest experiments with radioactive tracers in plants were confined to a study of the macroscopic distribution of certain substances, such as phosphorus, which could be readily labelled, and successful autoradiographs of leaves and fruits were obtained (Arnon, Stout, and Sipos 1940; Colwell 1942; Harrison, Thomas, and Hill 1944; Grosse and Snyder 1947). In the present investigation an attempt has been made to develop techniques which will increase the application of ^{14}C autoradiography to allow more detailed study of problems in plant and animal physiology. Low resolution autoradiography using X-ray film has yielded interesting qualitative information, and a simple method for the administration of $^{14}CO_2$ to plants during a period of photosynthesis has been used. Electron-sensitive emulsions have been employed to obtain increased resolution, and a method has been developed of measuring low concentrations of active isotope in tissue in which the electron tracks due to the specimen are counted. The problems of maintaining intimate association between specimen and emulsion and preventing isotope dissolution during tissue preparation and photographic exposure have been considered, and methods

* Botany School, University of Melbourne.

† Physics Department, University of Melbourne. Present address: Biochemistry Research Institute, Middlesex Hospital, London.

obtained which considerably reduce isotope displacement without loss of resolution.

Davenport and Stevens (1954), in a paper illustrating the use of X-ray film for comparing radioactivities, claim that by visual density matching, estimates of activities can be made with tolerable accuracy. In their report, the advantages of simplicity of the X-ray film technique and the possibility for practical comparisons of very weak sources are recognized. The possibility exists of describing the distribution of an isotope within whole leaves, stems, and roots, and of making estimates of the activities. Thickness of tissue must be kept in mind when comparing stem, root, and leaf autoradiographs, as comparisons can only be made between tissues of similar thickness.

Attempts have been made to obtain quantitative information from autoradiographs. The concentration of α-particle emitters in tissue has been measured by a count of α-particle tracks originating from tissue sections in contact with nuclear track emulsions (Endicott and Yagoda 1947; Rotblat and Ward 1953). The principal uncertainty in the technique appears to be the estimation of the self-absorption of the specimen. The method is one of high resolution, since the point of origin of the tracks may be observed with high precision. In autoradiography with β-emitting isotopes, quantitative estimates of the relative concentrations of the isotopes in different regions of the specimen have been achieved by a count of developed grains in the corresponding regions of the photographic emulsion (Andresen, Chapman-Andresen, and Holter 1952). Absolute determinations of isotope concentration by the method of grain counting have been made by Doniach and Pelc (1950), who also estimated the radiation dose received by the tissue in the course of the experiment, and by Nadler and Bogoroch (1951). The method is laborious and subject to errors arising from variations in the development conditions and from pseudo-photographic effects. Dudley and Pelc (1953) have developed an apparatus for automatic grain counting. This eliminates personal errors and allows a considerable reduction in statistical uncertainties. The apparatus projects a minute spot of light, moving on a pattern resembling a television raster, on to the autoradiograph. When the beam is interrupted by a silver grain an electric pulse is produced which is amplified and then passed through a discriminator unit before being recorded by a mechanical register. The number of grains rendered developable by the passage of a single electron through the emulsion must be known before the results of grain counts can be interpreted in terms of the actual concentration of active isotope present in the specimen. This can be achieved by standardization against a source of known specific activity, provided self-absorption is equal in both cases. Boyd and Levi (1950) have described a method of ^{14}C β-track autoradiography and they point out possibilities for quantitative evaluation of autoradiographs.

II. β-Particle Detection in Autoradiography

The observation of the macroscopic distribution of a radioactive tracer absorbed in plant tissue presents little difficulty. The tissue is placed against a

photographic plate, either in contact with the emulsion surface or separated from it by a thin protective layer, for a suitable period, after which the plate is developed and the image compared with the tissue structure. A convenient detector of the activity present is provided by X-ray film, which has a high sensitivity to β-particles and is easily handled and processed. Such film is not, however, well suited to the microscopic investigations of the distribution of an active isotope, since the large grain diameter, of the order of 2 μ, results in poor resolutions. Improved resolution can be obtained by the use of fine-grain emulsions, and in particular with nuclear emulsion, in which the reduction in sensitivity to be expected from the much smaller grain size (0·2-0·5 μ) is partially offset by the much higher concentration of silver bromide.

The resolution of an autoradiograph is not solely determined by the grain size of the emulsion used, but depends also on the specimen thickness, the separation between specimen and emulsion, and the β-particle range. The dependence of resolution on these factors is discussed by Doniach and Pelc (1950) and by Herz (1951). A lower limit of the order of 3 μ is to be expected for a sample thickness of 5 μ with an emulsion 2 μ thick.

If thick emulsion layers are used, in which the tracks of the individual electrons may be observed, comparable resolution may still be obtainable, since the point of origin of the tracks in the emulsion can be observed to an accuracy of the order of the grain diameter, even though the resolution of the image as a whole is lower than in the case of a thin emulsion layer. Full advantage cannot be obtained from observation of individual tracks unless the specimen remains in contact with the emulsion surface throughout the processing, so that the points of origin of the electron tracks may be directly related to the specimen structure.

The electron-sensitive emulsion now available can record the passage of electrons at minimum ionization energy (0·5 MeV), which produce tracks of approximately 30 developed grains per 100 μ path length. The tracks of high energy electrons are practically linear, but as the energy decreases, large-angle scatters become increasingly frequent and the greater ionization results in an increase in the number of grains per unit path length.

Since electron-sensitive emulsions record the passage of every ionizing particle they rapidly accumulate tracks due to cosmic radiation and local radioactive contamination. The rate of formation of such tracks in an unshielded emulsion, 200 μ thick, is of the order of 200 per sq.mm. per day. This track background, which is superimposed upon the image produced by the specimen, is frequently quoted as an objection to the use of such plates for autoradiography. It does not, in practice, limit the detection of low levels of activity too seriously if freshly prepared plates are used, since specific activities of the order of 10^{-9} c/g would yield electron tracks at a rate comparable with the formation of background tracks. If necessary the background accumulation rate can be reduced by shielding. If the plates are enclosed with a 2 in. lead wall and covered by a thickness of 4 in. of lead during the exposure, the background will be decreased by a factor of about nine (Fremlin and Walters 1950).

One of the advantages to be gained from the use of thick electron-sensitive emulsions is that quantitative estimates of the concentration of active isotope in the specimen may be made from a count of the electron tracks forming the autoradiograph. In such measurements the background of tracks due to other sources must be observed in a region of the plate protected from the active specimen, and the measured value of the sample emission corrected accordingly. Tracks caused by the emission of electrons from the specimen are easily distinguished from fog grains and pseudo-chemical effects.

Electron tracks, particularly those originating from an isotope of low disintegration energy, are not easy to count as a consequence of the wide variation in initial energy of the electrons producing them, and the frequency of large-angle scatters. If, however, the total number of tracks in 1 sq. mm. of the autoradiograph is kept below 500, and the tracks are observed with high power, oil immersion objective, repeatable counts may be made. The counting is simplified if only those tracks intersecting the upper emulsion surface are included in the total. This also effectively reduces the track background, since many background tracks are caused by electrons arising from the absorption of γ-radiation within the emulsion or glass backing, and do not penetrate to the emulsion surface.

In order to obtain an absolute estimate of the specific activity of the sample from the results of such a count, several factors such as self-absorption in the source, back-scattering in the emulsion, statistical uncertainties, and observational errors must be taken into account. The possibility of calibrating the detection system exists for the case of ^{14}C since A.E.R.E., Harwell, supply standard sources of polymethylmethacrylate sheet containing accurately measured concentrations of ^{14}C. These may be sectioned to the same thickness as the specimen. Counts of tracks produced by such a source allow the combined effects of self-absorption, back-scattering in the emulsion, and any failure to include the tracks of very low energy electrons in the count to be determined. The number of electron tracks observed within a definite area of an autoradiograph may then be related to the specific activity of the sample by the application of the laws of radioactive decay. If the isotope concerned has a half-life $T_{\frac{1}{2}}$, long in comparison with the exposure period t, the total number of electrons emitted n, is given by the relation

$$n = N \lambda t,$$

where N is the total number of radioactive atoms present in the sample, and λ is the decay constant of the isotope and is equal to $0{\cdot}69/T_{\frac{1}{2}}$.

Only a certain fraction of the electrons emitted by the active isotope will enter the emulsion, this fraction being determined by self-absorption in the specimen and the geometry of the system. If a specimen, thin in comparison to the β-particle range, is exposed in contact with the emulsion surface, it may be assumed that one-half of the emitted electrons will be recorded in the emulsion. An error of less than 5 per cent. is involved for a sample thickness of one-tenth of the β-particle range. If a tissue density of 1 is assumed, a specimen 40 μ thick would allow half of the β-particles emitted to pass out of the tissue. As sections 5 and 7 μ thick are used it may be assumed that the

error due to self-absorption is small. When thicker sources are used, self-absorption becomes appreciable. The number of electrons which are able to reach the surface of a thick source of known specific activity has been estimated by Libby (1947) on semi-empirical grounds. Using the experimental fact that electrons from typical β-emitters are absorbed exponentially up to 90 per cent. loss of intensity, it is shown that the number of electrons reaching the surface of a source of infinite thickness is

$$I = \frac{A \sigma L_0}{2 \times L_0^a},$$

where I = intensity,
A = area of source,
σ = specific activity,
L_0 = β-particle range, and
a = absorption coefficient of the β-particles in the material of the source.

For many β-emitters the product $2 \times L_0^a$ is approximately equal to 10. Thus one-tenth of the β-particles originating in a layer of the source of thickness equal to the range are able to reach its surface.

For sources of thickness x (less than the β-particle range), the intensity I_x at the surface is related to the intensity I_∞ due to a source of thickness equal to or greater than the range by the expression

$$I_x = I_\infty \ (1 - e^{-ax}).$$

Values of the ratio I_x/I_∞ for different source thicknesses, expressed as a fraction of the β-particle range, have been calculated and are shown graphically in Figure 1.

The proportion of the disintegrations likely to be absorbed in any practical case may thus be estimated if the source thickness and β-particle range are known. Absolute measurements of the concentration of radioactive tracers in tissue may, therefore, be made with reasonable accuracy if thick nuclear emulsions are employed, the accuracy of the determination being limited primarily by uncertainties as to the amount of self-absorption occurring in the source and the detection efficiency. Statistical uncertainties may be reduced to a value small in comparison with other errors without undue effort. The method is one of very high sensitivity, a concentration of ^{14}C of only 5×10^{-10} g per g of tissue being readily detectable with unshielded plates.

III. Experimental Procedure

(*a*) *Administration of $^{14}CO_2$ to the Plant*

$^{14}CO_2$ is introduced to the plant in photosynthesis, and suitable exposure apparatus is required for the control of this process.

One administration method allows control of the site of $^{14}CO_2$ assimilation and the use of intact plants. This requires a 10 c.c. weighing bottle with a sq.cm. opening at the bottom. The exposure chamber is attached to the abaxial surface of the leaf with porometer luting wax applied to the ground edge of the open square. Before attachment the required weight of

$BaCO_3/Ba^{14}CO_3$ (1560 : 1) is placed in the inverted weighing bottle to give a known total concentration of CO_2. When adding carrier to obtain the required proportion great care must be exercised to obtain a uniform mixture. This has best been achieved by mixing in a small amount of carrier at a time and grinding the two together with a glass rod on a ground glass surface. At the commencement of the administration period, 0·2 ml (an excess) of N HCl is injected with a hypodermic syringe on to the $BaCO_3$ liberating a $CO_2/^{14}CO_2$ mixture (see Plate 1, Figs. 1 and 2). The abaxial side of the leaf is sealed on to the exposure chamber with the wax previously applied, and the

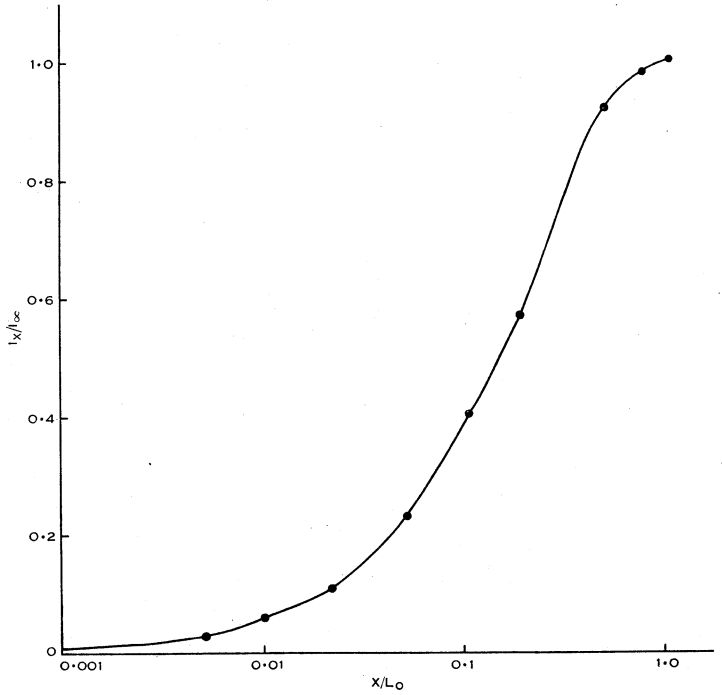

Fig. 1.—Values of the ratio I_x/I_∞ for different source thicknesses, expressed as a fraction of the β-particle range.

plant is allowed a period for photosynthesis. Most experiments were conducted in light intensities varying between 500 and 2000 f.c. At the end of the experiment the leaf is lifted quickly from the exposure chamber and replaced by a small piece of paper. In this way the amount of active gas escaping to the surrounding atmosphere is kept at a minimum. The plant is then dried in preparation for placing in contact with the photographic emulsion.

(*b*) *Tissue Fixation and Dehydration*

(i) *For Macroscopic Autoradiography.*—For the preparation of small tissue samples (i.e. a single leaf or seedling) used in the determining of macroscopic

distribution of ^{14}C, initial freezing is achieved by the immersion of tissue in an alcohol-dry ice freezing mixture, or for leaves, insertion in a Dewar flask containing crushed dry ice. Once frozen, the tissue is placed over a concentrated sulphuric acid trap in a vacuum desiccator attached to a high vacuum pump. A small quantity of dry ice is placed with the sample in the vacuum desiccator to prevent thawing before a high vacuum develops. This system achieves a pressure of 0·1-0·4 mm Hg, which gives satisfactory drying in 36-48 hr. Absolute dryness is not achieved by this procedure, but the moisture content of the tissue is reduced to a level satisfactory for X-ray autoradiography, and movement or displacement of isotope is insignificant when considered in relation to the resolving power and other limitations of macroscopic autoradiographs. When movement of the isotope into a particular tissue, as opposed to movement within a tissue, is the object of the experiment, air drying after dissection of the plant is satisfactory. Other methods of drying, including heat treatment of the tissue, cause considerable movement of the isotope in the vascular system of leaves and stem.

In the preparation of autoradiographs, the dried leaves, stems, and roots are placed on sheets of paper 5 × 7 in., their outlines traced, and other identification details noted (Plate 1, Fig. 3). Then the paper with specimens is placed between two sheets of ⅛ in. glass of the same dimensions. Finally, in the darkroom, using a Wratten No. 1 filter, a piece of "Kodirex" (no-screen) duplitized X-ray film is placed in contact with the tissue, and the whole held firmly with spring-back clips. These plates are then deposited in a light-tight tin which is kept at 0-1°C for 14 days. Variation of the exposure time presents a useful possibility for the comparison of tissue autographs and for activity estimations. Development is with Kodak X-ray developer for 3 min at 20°C, and the acid fixing solution, described below (see Appendix II(*b*)), is used after dilution with an equal volume of water. Contact prints are made from the autoradiographs, using light-weight glossy paper of high contrast, exposed 5 ft from an unshaded, frosted, 300-W, incandescent globe for 1-3 sec. Improved quality of autoradiograph prints can be achieved with photographic reduction.

(ii) *For Microscopic Autoradiography.*—Freeze-drying apparatus is used in tissue preparation for the purpose of obtaining autoradiographs of histological sections. The unit designed by Moberger, Lindstrom, and Andersson (1954) requires an oil diffusion pump and mechanical backing pump to reach a pressure of 10^{-4}-10^{-5} mm. Hg. It is found that satisfactory preservation of plant tissue structure can be achieved with a simpler and less expensive design. The two vacuum pumps are replaced by a "Dynavac 6W" pump with a thermal stripping attachment, which prevents emulsification of the pump oil by water vapour from the specimen. Thus a water trap for the protection of the pump is not required. This pump will give a pressure of 10^{-3} mm Hg.

The tissue is placed on a piece of wire gauze supported by two blocks of degassed paraffin. Moberger *et al.* (1954) used an oscillator to heat the tissue holders within the tissue chamber, while it is found in this Laboratory that an infra-red lamp gives close control of paraffin melting. Well-preserved

tissue has been obtained without using a liquid air cold finger, but improvement might result from its inclusion, and drying time would doubtless be reduced. Greater detail of this method is to be published elsewhere. Immediately following exposure to the isotope, small tissue samples (total fresh weight not exceeding 100 mg) are quenched by direct immersion in liquid air, precooled *iso*pentane, or liquid propane. Specimens are then quickly transferred to the detached tissue chamber, which is a rigid fixture in a Dewar flask filled with alcohol-dry ice freezing mixture. The tissue chamber is fitted to the vacuum system and the pump switched on. Initial drying at a temperature lower than $-60°C$ is allowed to continue from 6 to 12 hr, and then the Dewar flask is removed and the temperature of the tissue chamber allowed to equilibrate with air temperature. Pumping is continued for a further 12 hr. At the end of this period an infra-red lamp is brought in close proximity with the tissue chamber until the paraffin, above which the specimen has been placed, begins to melt, causing the collapse of the specimen stage and immersion of the specimen in the paraffin wax. The vacuum may now be broken and the impregnated specimen removed. The tissue at this stage is extremely brittle and must be handled with care. Paraffin blocks are trimmed to the required shape and size and microtome sectioning carried out in the usual manner. Sectioning should not be attempted until the photographic emulsion is poured and dried. The time lapse between section cutting and application to the emulsion surface must be kept to a minimum.

Hand sectioning of fresh material is a useful method of histological preparation. Stem sections are cut with a safety razor-blade in the darkroom and placed directly on to the emulsion surface. Good adhesion between the emulsion surface and the specimen is obtained without excessive chemical damage to the emulsion. An alternative would be to float the hand sections of living material on water and transfer these to the emulsion surface in the darkroom. Chemical fogging, if this occurs, is immediately distinguishable from the autoradiograph, which consists of β-particle tracks passing through the emulsion.

(*c*) *Spreading Paraffin Sections*

It has already been stated that one of the first requirements for the success of the microscopic technique is the prevention of isotope loss. Freeze-drying and paraffin embedding under vacuum give a ready means of tissue fixation with the possibility of only slight losses of lipoidal materials, but the spreading of sections presents a greater problem.

Sections $7\,\mu$ thick are floated on a glycerine surface on a standard microscope slide, which is placed on an asbestos sheet over a water-bath (at approx. 40°C). Wetting of sections is kept to a minimum. The paraffin ribbon is divided into pieces containing two sections each, and the warmed slide transferred to the microscope stage and sections examined under the high-power lens. This examination allows an assessment of the quality of the section and cools the paraffin, which is necessary before further manipulation is possible. Once cool, the sections can be removed from the glycerine surface with the

aid of a camel-hair brush. This operation is not simple and will require practice before removal can be accomplished without damage to the sections. The aim is to remove the section with little or no glycerine adhering to its surface. Sections are placed on filter paper and the remaining free glycerine removed.

The glycerine film remaining is sufficient to hold the sections firmly on the emulsion surface, and when lightly wiped with a dry camel-hair brush provides an even and intimate contact throughout the exposure period. After photographic exposure, the plate is immersed in distilled xylol for approximately 2 min to remove paraffin from the sections before development. Using this method undamaged sections have adhered to the emulsion surface throughout photographic processing. Adajacent sections mounted in the usual way may be stained if greater detail is required for comparison with the section used to produce the autoradiograph. The staining of sections mounted on processed emulsion is possible, using alcoholic stains, but no great advantage results for leaf tissue. An experimental evaluation of isotope loss and displacement is presented in Appendix I.

(*d*) *Photographic Processing*

The electron-sensitive plates used in this investigation have been prepared in the laboratory, immediately prior to use, from G5 gel supplied by Ilford Ltd., England. Plate pouring avoids the unnecessary accumulation of background tracks, which would otherwise reduce the sensitivity of the method. The glass surface is first coated with a thin layer of a 5 per cent. solution of gelatine in water, which is poured at a temperature of 50°C over the glass supported on a cool, levelled table. When dry, the plate, a No. 1 coverslip 1¼ × ⅞ in., is coated with G5 gel under a Wratten No. 1 safelight. The emulsion is warmed to a temperature of 55°C on a water-bath and 0·5 c.c. of the gel delivered to the surface of the plate with a warm, hypodermic syringe with no metal parts. An emulsion thickness of approximately 50 μ results. When set, the plate may be moved and dried in a stream of warm air at a temperature not exceeding 37°C. The best plates have been obtained when the humidity of the drying room was high, and the drying period extended over 12 hr. The thin gelatine layer is found to increase the adhesion between the glass and the emulsion, and to reduce reticulation during later processing. The coverslip may be more manageable if attached to the surface of a microscopic slide with a drop of water.

The development of nuclear track emulsions differs from that of optical emulsions, partly because of the greater concentration of silver bromide in the emulsion, and partly because the grains in which the latent image is formed are within and on the surface of the silver bromide crystals. An amidol-borax developer of the type described by Dainton, Gattiker, and Lock (1951) is used (see Appendix II(*a*)), and the "temperature development" method of Dilworth, Occhialini, and Payne (1948) employed in the case of emulsions of thickness greater than 50 μ, or on those sections which adhere to the emulsion surface. In this method the plate is soaked in the developer at a temperature below 5°C for a period of 3 min, during which time the developer penetrates

throughout the emulsion layer but the reaction is considerably retarded. The plate is then taken out of the solution, surplus developer removed from its surface, and placed glass downwards on to a hot plate at a temperature of 20°C for 20 min. Development then proceeds uniformly throughout the layer. On completion of the development the plate is transferred to an acid fixing bath (see Appendix II(*b*)) at 5°C, which terminates the development reaction while allowing the fixing solution to penetrate the emulsion. We have found it necessary, with plates prepared in this Laboratory, to complete fixation and washing at this low temperature in order to avoid reticulation. Acid fixing is continued for double the time taken for the plate to clear at 0-1°C. Washing is conducted at the same temperature, and both acid fixer and washing water are pre-cooled to approximately 5°C. Washing is continued until a sample of the water no longer reduces (pink to brown) potassium permanganate when a drop of a 1 per cent. solution is added. The plate is dried in a horizontal position in air, or dehydrated in a series of alcohol-water solutions of increasing alcohol concentration. The second method of drying is preferable in the case of plates carrying sections which are subsequently stained, since it results in more complete dehydration of the emulsion.

When dried, the coverslips, with emulsion and specimen attached, are inverted on to a drop of ethyl alcohol (previously dried with anhydrous copper sulphate) on a microscope slide. Using the oil immersion objective the β-particle tracks recorded in the emulsion can be associated with the specimen. With downward focusing the tracks are seen first and then the tissue.

IV. Illustrations of Macroscopic and Microscopic Autoradiographs

This study was embarked upon with the primary purpose of finding a satisfactory experimental method for the study of translocation of organic materials in plants. The combination of macroscopic and microscopic autoradiography allows the record of the movement of assimilated isotope, and the results of experiments using these methods are to be published. Autoradiographs are presented here only to give illustration of the methods.

The G5 autoradiographs (e.g. see Plate 2, Fig. 6, and Plate 3, Fig. 7) illustrate the order of accuracy with which the activity can be located. The majority of sections remain undamaged throughout the photographic processing, and this arrangement allows detailed association of tissue structure with β-track origin at the emulsion surface. If the unstained section adhering to the emulsion surface is immersed in a drop of ethyl alcohol under a No. 1 coverslip, both section and autograph may be seen with a $\times$ 90 oil immersion objective. Since, in the majority of cases, tracks enter the emulsion fairly steeply, only the first few grains are in focus in the photomicrograph. Single fog grains, which are readily distinguishable from tracks under working conditions, may be confused with the origin of β-particle tracks when viewed at a single focusing level; the photomicrographs, in which the tissue is slightly out of focus, are presented to illustrate the technique.

Plate 1, Figure 4, is a phase-contrast photomicrograph (magnification $\times$ 1300) of the leaf cells and part of their autoradiographs. It is clear that very

close correlation between the tissue and emergent β-particle tracks is possible with this technique. A spongy mesophyll cell (X) in the centre of Plate 1, Figure 4, gives rise to five tracks originating in the centre of the cell and two tracks (of low energy and at a wide angle) from the cell wall. The disturbance of cell contents caused by photographic processing prevents the association of tracks with the structural features of the cells.

Plate 1, Figure 5, is presented to illustrate the record obtained of isotope distribution in G5 autoradiography, which immediately discloses if the isotope has moved from the tissue during preparation. From this autoradiagraph of a transverse section of a *Cucurbita* petiole it can be seen that the isotope has moved into the embedding paraffin along the margin of the section, and it is reasonable to believe that similar movement occurred within the section. It is clear that such experimental errors are immediately evident, an attribute which aids accurate interpretation of the autoradiograph. This autoradiograph also illustrates that at lower magnification ($\times$ 480) autoradiographs containing too many tracks for counting may be visually estimated, and so provide valuable information concerning the relative activities of different tissue regions. In the freeze-drying and subsequent processing, tissue damage has occurred and none of the cellular contents have been retained, but by using more efficient freeze-drying apparatus complete tissue preservation has been achieved.

Satisfactory microscopic autoradiographs have been prepared by cutting hand sections of fresh tissue containing assimilated ^{14}C. These fresh tissue sections are placed directly on to the surface of the G5 emulsion previously poured on to a coverslip. The objection to this method of tissue preparation is the possibility of redistribution of the isotope during sectioning. However, this possibility can often be ruled out because of the pattern and distribution of isotope recorded in the autoradiograph. The preservation of tissue structure and contents is satisfactory, and the use of hand sections should provide a useful application of the microscopic technique. Illustrations of the autoradiographs obtained from hand sections are presented in Plate 2, Figure 6 and Plate 3, Figure 7.

X-ray plate autoradiographs can record isotope distribution in whole plants and should, therefore, prove useful in the study of correlative nutritional relationships existing between plant organs. An experimental method is thus provided for the study of translocation of nutrients. Mineral uptake and distribution in plants may also be investigated using this method of autoradiography.

Examples of the macroscopic autoradiographs obtained are shown in Plate 3, Figures 8 and 9. In Plate 3, Figure 8, the movement of ^{14}C from the site of $^{14}CO_2$ administration in the leaf of soya bean up the stem to the apex and expanding leaf is recorded. No detectable autoradiograph is given by the opposite primary leaf, which is outlined to indicate its position on the X-ray film. The root autoradiograph (Plate 3, Fig. 9) shows accumulation of ^{14}C at the root tips, illustrating that the isotope incorporated in the products of photosynthesis moves from the illuminated primary leaf to these sites of meristematic activity and growth.

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Explanation of Plates 1-3

Plate 1

Fig. 1.—Exposure chamber showing ground glass edge.

Fig. 2.—Exposure chamber showing position of leaf on exposure to $^{14}CO_2$ during photosynthesis.

Fig. 3.—Arrangement for X-ray plate exposure.

Fig. 4.—Autoradiograph of several spongy mesophyll cells. Seven β-particle tracks are associated with the cell *X* in the centre of the photograph. Phase-contrast. × 1300.

Fig. 5.—Autoradiograph of a transverse section of a *Cucurbita* petiole produced from a 48-hr exposure. Note loss of isotope which occurred during embedding due to overheating paraffin. Tissue distortion and loss of cell contents are also evident. × 480.

Plate 2

Fig. 6.—Autoradiograph of a longitudinal section of a soya bean stem. A pathway of active material across the sieve tube (*X*) can be detected from the line of β-particle tracks recorded in the overlying emulsion. Disturbance of the contents is indicated by the conglomerate in the centre of the sieve tube. β-particle tracks are also associated with the contents of the phloem fibres (*Y*). Phase-contrast. × 670.

PLATE 3

Fig. 7.—Autoradiograph of a longitudinal section of the phloem of soya bean. A pathway of active material across the sieve tube (X) can be detected from the line of β-particle tracks recorded in the overlying emulsion. It will be noted that phase-contrast microscopy, used in Plate 2, Figure 6, gives improved association between the tissue and the autoradiograph. Oil immersion. × 1500.

Fig. 8.—Autoradiograph recording the site of isotope administration, the pathway, and sites of accumulation of the products of photosynthesis, resulting from an exposure of the primary leaf of soya bean to $^{14}CO_2$ for 3 hr. × 1/3.

Fig. 9.—X-ray film autoradiograph of part of a soya bean root following $^{14}CO_2$ administration to a leaf. × 1/3.

APPENDIX I

(*a*) *Isotope Loss and Displacement*

Because of the basic importance of isotope loss or displacement to the success of the method, several plates were prepared for the measurement of activity levels as influenced by the time sections remained on a glycerine surface. Sections 7 μ thick were cut from the tip of a soya bean leaflet, and exposed to the nuclear emulsion for 144 hr. Table 1 gives results of track observations.

TABLE 1

INFLUENCE OF TIME OF SECTION ON GLYCERINE SURFACE ON TRACK DISTRIBUTION

Section No.	Time on Glycerine (min)	No. of Sections	No. of Fields	No. of Tracks per Field	Comment
1	2	1	15	22·6	
2	4	1	10	24·2	
3	4	1	10	22·2	Warmed twice
4	6	1	16	20·3	
5	6	1	10	23·3	Warmed twice
6	8	1	10	24·1	Warmed twice
7	8	1	10	25·6	
8	10	1	15	31·6	Warmed twice
Background			200	0·25	
4	6	1	33	0·79	Marginal
Mean per field				23·9	

The 33 fields around the margin of section No. 4 were counted for comparison with the background count. There are more tracks in this region than in the background, but the increase is small in comparison with the activity of the section. An increase of this magnitude could be accounted for by a residue of either adsorbed ^{14}C or isotopic lipoidal materials or both being displaced during paraffin embedding. The possibility remains that trace amounts escape from the section.

It can be seen from Table 1 that the track count does not decrease with time on glycerine over a 10-min period. Sections warmed twice do not show

a lower activity. On the contrary, there is a gradual rise of activity recorded for sections Nos. 5 to 8 inclusive, and section No. 8 shows a considerable increase. This rise recorded for section No. 8 is a conservative value, for in some areas of this section tracks were too numerous to count. The result suggests a displacement of ^{14}C within the section, when placed on a glycerine surface for 10 min and warmed twice, but evidently loss of ^{14}C to the glycerine does not occur.

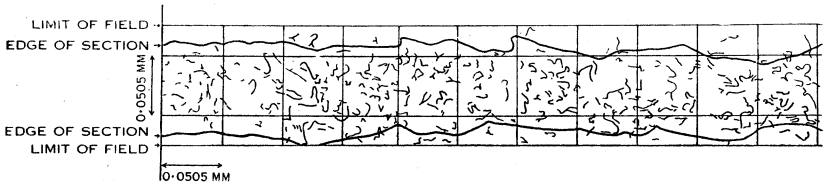

Fig. 2.—Distribution of β-particle tracks originating from a transverse section of soya bean leaf spread on anhydrous glycerine for 8 min and warmed twice.

A graphical picture of ^{14}C distribution within the soya bean leaf is shown in Figure 2. Relatively uniform distribution in mesophyll cells provides evidence that isotope displacement has not occurred. An uneven distribution would be expected if active materials had been moved from one part of the section to a new site, and such distribution was observed in section No. 8. The

TABLE 2

TRACK OBSERVATIONS TESTING THE CONSISTENCY OF TRACK COUNTING IN 16 SERIAL SECTIONS

Section	No. of Fields Observed*	Mean No. of Tracks Observed†	Section	No. of Fields Observed*	Mean No. of Tracks Observed†
A	55	1·8	J	18	2·0
B	20	1·7	K	13	1·4
D	19	1·3	M	54	1·3
E	21	1·7	N	29	1·2
F	17	1·5	O	24	1·1
G	23	1·8	P	57	1·3
H	60	1·3	Q	25	1·7
I	31	1·3	R	15	1·7
			‡Background	100	0·03

* Total number of fields observed = 481.
† Mean number of tracks per field = 1·5.
‡ Equivalent to 84 tracks per sq. mm.

small areas where activity is noticeably absent correspond to xylem tissue areas in that section. No tracks have been observed associated with xylem vessels, which again suggests that the isotope has not been displaced during processing.

To test the consistency of results that can be obtained from track counting when a very low concentration of ^{14}C is present in the tissue sections, a plate

was prepared from a masked region in the middle of the soya bean leaflet used previously. Of the 18 sections placed on the emulsion surface, 16 remained after processing. The damage to remaining sections varied, and the extent of this damage is indicated by the number of fields counted. The plate was exposed to sections for 92 hr. The result of this track counting is presented in Table 2.

The mean of such a large number of relatively uniform observations can be accepted as a valid representation of the ^{14}C present in the tissue.

When the activity of the two tissue samples exposed to $^{14}CO_2$ in the light (tip and base of leaflet) are compared on the basis of equal G5 exposure time, their ^{14}C concentrations fall within the same order of magnitude (i.e. base 17·2 and tip 23·9 tracks per 60 μ^2). The apparently higher concentration in the leaf tip may be explained by the smaller area occupied by the xylem in this tissue sample. Compared on the same basis the tissue exposed to ^{14}C in the dark shows ^{14}C activity of 2·2 tracks per 60 μ^2.

It is concluded that there is little or no loss or displacement of the ^{14}C isotope caused by spreading sections on anhydrous glycerine at 40°C for 2 min, and that, under the conditions of this experiment, similar concentrations of ^{14}C occur at the tip and the base of the Biloxi soya bean leaflet separated by a masked region after 1½ hr of photosynthesis in an atmosphere containing $^{14}CO_2$.

Appendix II

(*a*) *Amidol-Borax Developer*

The composition of the amidol-borax developer is as follows:

Amidol	1·12 g
Sodium sulphite, anhydrous	4·5 g
Sodium sulphite, crystalline	9·0 g
Boric acid	8·75 g
Potassium bromide, 5 per cent. solution	4·0 ml
Water	to 250 ml.

The sodium sulphite and boric acid are dissolved in 130 ml of distilled water at 50°C, then the amidol (dark crystals containing the product of oxidation are avoided) is dissolved in 80 ml of cold distilled water. The amidol solution is quickly filtered through cotton wool and the two parts mixed. Finally, the potassium bromide solution is added and made up to volume with distilled water. The amidol solution must not be allowed to discolour, otherwise stained plates and defective developing will result. This developer stored at 1°C can be used for 3-4 days after preparation.

(*b*) *Acid Fixing Solution*

The composition of the acid fixing solution is as follows:

Sodium thiosulphate	400 g
Sodium bisulphite	30 g
Water	1000 ml.

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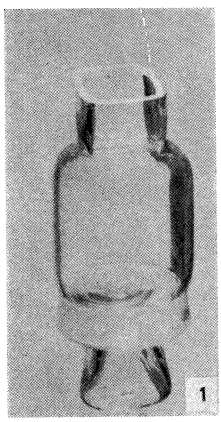

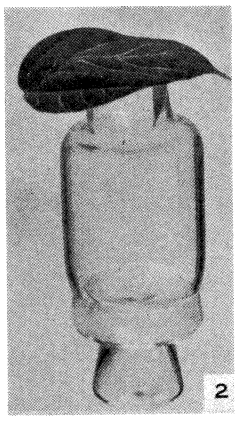

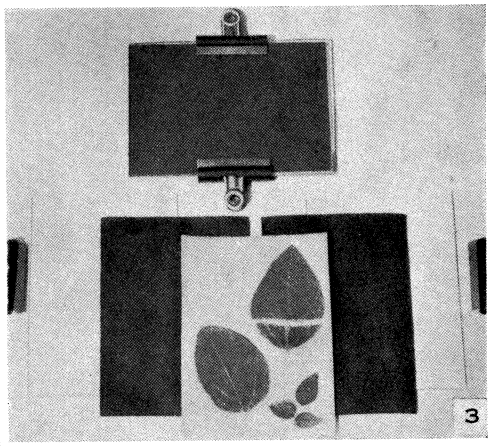

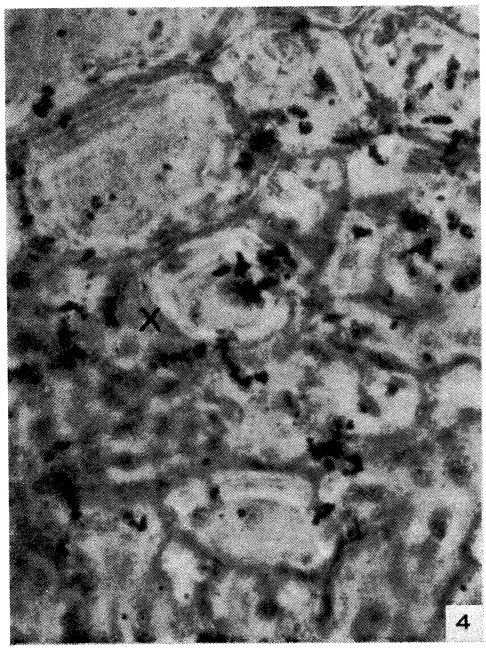

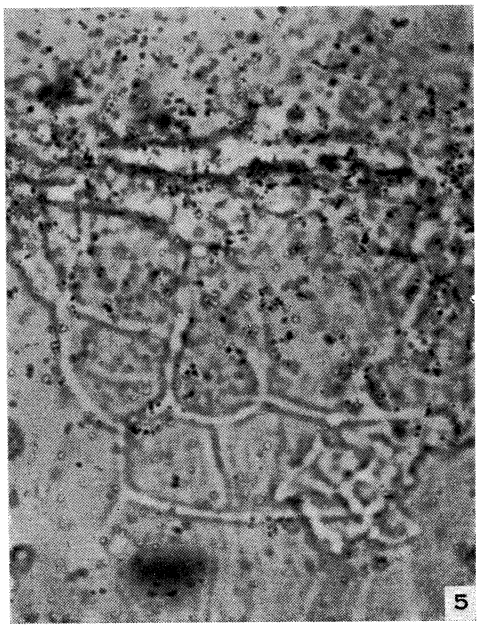

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