A RAPID METHOD FOR LOCATING AND ELUTING RADIOACTIVE STREAKS ON AN ELECTROPHEROGRAM OR A CHROMATOGRAM

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INTRODUCTION

During the course of our studies on the relative stability of short lived ultraviolet (U.V.) photoproducts of cytidylic acid and related compounds[1,2] we were faced with the difficult problem of locating the separated products on an electropherogram quickly and with enough precision so that they could be isolated and eluted for further kinetic studies.

The standard technique of locating products with a U.V. lamp (Mineralite) is often not satisfactory because many of the products one wishes to locate have no U.V. fluorescence or absorption. If the material is radioactive the spots can be located by autoradiography, but this is of no value if the materials are unstable and change chemically with a short half life.

A two dimensional scanner for radiochromatograms which used a single Geiger Mueller end window counter has been described by ARNOFF[3]. In his device a stylus was used to record data on electrosensitive paper. GILBERT AND KEENE[4] developed a linear array of counters to detect without scanning the radioactive distribution along a chromatographic strip. Such a device has a resolution of about 1 cm corresponding to the diameter of the detector. In their device the pulses from the counters were used to charge an array of condensers and the net charge on each recorded after a given interval.

Both these designs are suitable for detecting chemically *stable compounds, well separated* on a radiochromatogram.

In this paper we describe the design of a scanner which combines the advantages of using a multidetector and a recording technique similar to that described by ARNOFF. The additional features include use of an adjustable collimator for improving resolution and facilities for maintaining the compounds at 0° during the scan. Using our scanner all the radioactive spots on a large electropherogram (15 × 30 cm) can be located in about five minutes. Following this location the spots can be quickly eluted by a technique which will be described. With the special electrophoresis apparatus which we have developed[1] and the scanner and elution technique described here, it is possible to separate photoproducts, locate the spots containing them and elute them in less than 30 min all the while maintaining the materials at 0°.

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DESCRIPTION OF SCANNER AND ELUTION TECHNIQUE

Fig. 1 is a photograph of our rapid radioisotope scanner. It consists of a fixed detector assembly together with its collimator and shield, a mechanical scanning device which moves the electropherogram under the detector assembly and a data recording and display unit together with the associated electronics. These components will now be discussed in detail.
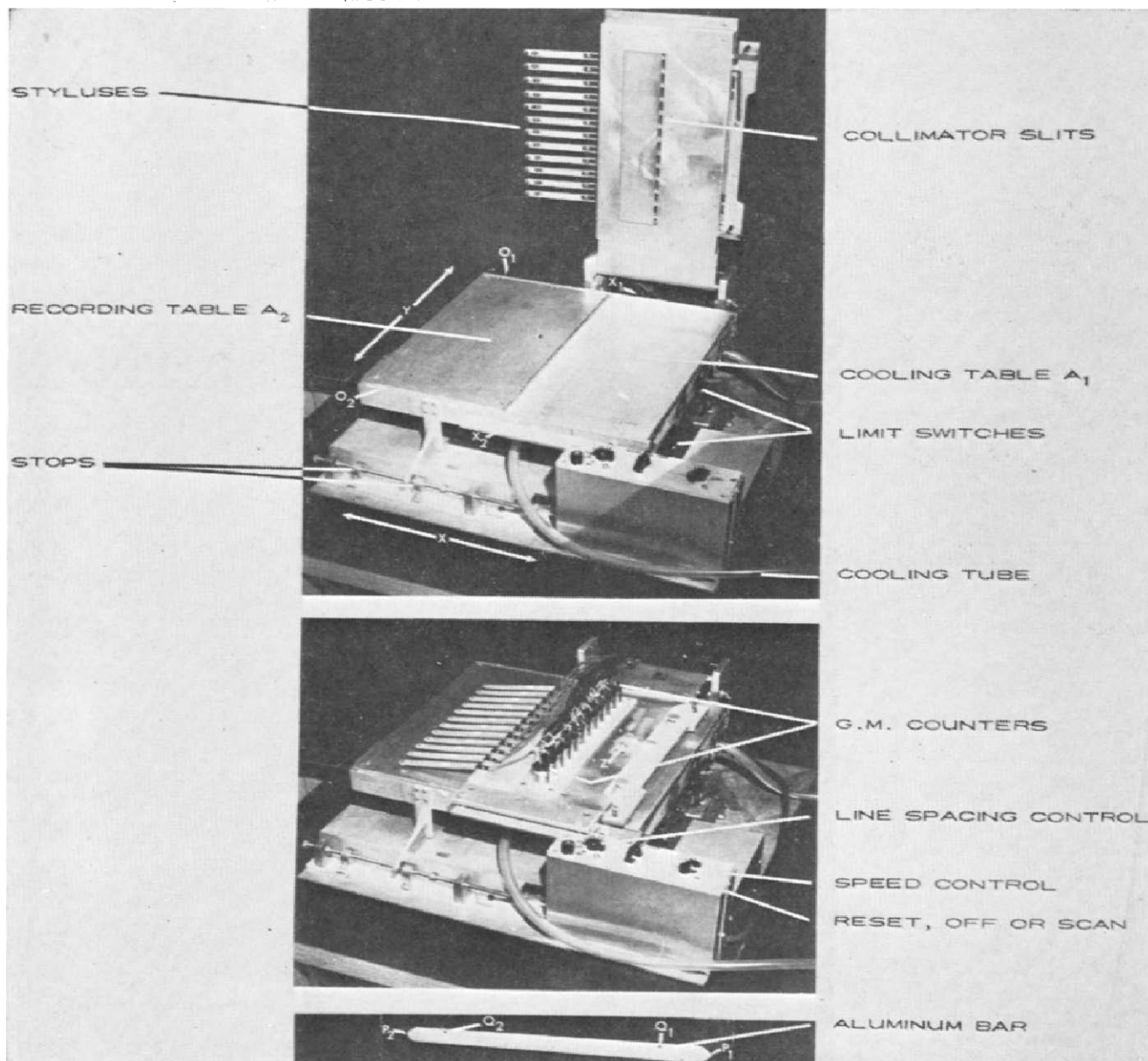

Fig. 1. Scanner with collimator assembly tipped away from table A₁ is shown on the top. The figure in the center shows the collimator assembly set up for scanning; the bank of G.M. counters can be seen above the collimator. The bottom figure shows the aluminum bar used for locating the products on the electropherogram from the corresponding dot scan.

Detector, collimator and shield

A bank of 12 Geiger Mueller (GM) end window halogen counters (Philips 18505) are used as the radiation detectors (Fig. 1). These have a good plateau (450–700 V)

with a slope of 2 %/100 V, a low background (15 c.p.m.) and 100 % efficiency for the detection of ^{32}P β rays. These 12 counters are mounted 2.5 cm apart on a 1.6 mm brass sheet placed over two layers of aluminum sheet as shown in Fig. 2. The counter is separated from the electropherogram by an adjustable slit (Fig. 2a) whose width can be adjusted from 0 to 6 mm by turning the screw Z shown in Fig. 1. In order that beta particles enter the sensitive region between the central electrode and the wall, the slit is off set from the centre of the GM counter (Fig. 2). For convenience in positioning the wet electropherogram on the table, the detecting assembly is hinged so that it can be tipped away from the table A_1 as shown in Fig. 1 (top).
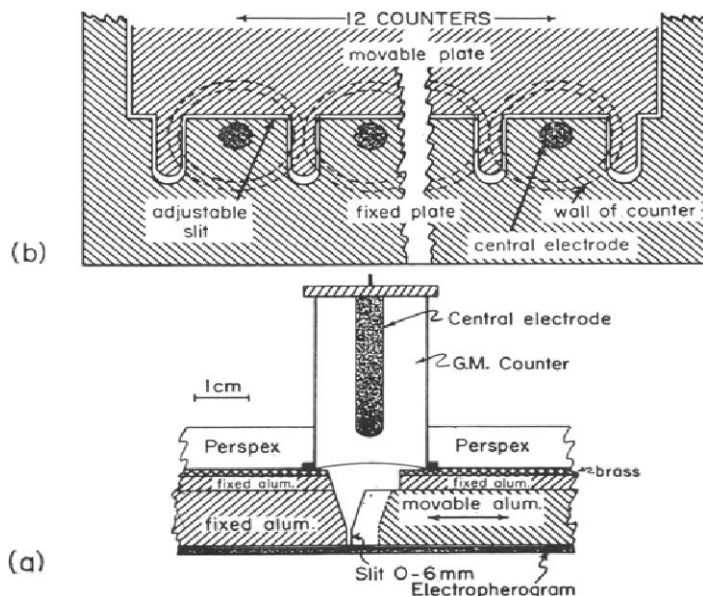

Fig. 2 (a) Section of collimator showing adjustable slits. (b) Section looking from above showing G.M. counters.

Fastened to the Perspex sheet are 12 styluses (Fig. 1) which are spaced exactly the same as the counters and project 9 in. to the left. Each stylus is connected to a counter through a simple electronic circuit to be described later. Every particle which enters the counter produces a spark between the corresponding stylus and the recording table A_2 (Fig. 1), making a mark on the electrosensitive paper (teledeltos-Western Union Telegraph Co., N.Y.).

Mechanical scanning device

The basic unit for the scanning device is a commercially available positioning table (No. 92-14, 'Palmgren', Chicago Tool and Eng. Co. Chicago, Ill., U.S.A.), with two screw fed slides mutually at right angles. Mounted on this table is a hollow ice water cooled plate A_1 on which is placed the wet electropherogram immediately after completion of electrophoresis. Adjacent and connected to A_1 is mounted a second plate A_2 which is thermally insulated from A_1 and on which is placed the teledeltos paper. Plates A_1 and A_2 move under the styluses and counters in the X direction a distance adjustable and up to 14 cm and in the Y direction 2.5 cm. These motions are produced by two independent motors.

The X direction corresponds to the direction of the electric field in the electrophoresis apparatus or the direction of flow of the solvent on a chromatograph. The

distance of scan in the X direction is adjusted to cover the region which includes the photoproducts of interest. The X motor is a Universal type (a.c./d.c., 1 A 110 V) which moves the table at velocities from 0.06 to 1 cm/sec. At the end of the scan the X motor stops and the Y motor (Bodine type NS1-12R, split phase 110 V, 60 cycles) is turned on for a predetermined time to move the table from 0.1 to 0.02 cm (giving line spacings from 10 to 50 lines per cm) and the X motor restarted in the reverse direction. Once the scan is complete the tables can be returned to their start positions by pressing a reset button. Thus after a complete scan each counter actually moves over an area of 14 cm × 2.5 cm and the total area scanned by the 12 counters is about 14 × 30 cm². The total length of time can be varied from 4 min to 250 min. In practice 5 min scans give all the detail one requires.

If a much smaller area of the electropherogram is of interest the length of the scan can be reduced and a detailed pattern of it obtained in a shorter time.

Electronic circuits

Negative pulses from the Geiger counter are differentiated and fed into the 6 BC 5 (see Fig. 3) giving a positive pulse of about 15 V which triggers the thyratron (2D21) allowing the 0.5 μF condenser to discharge through the primary of the auto-transformer. The autotransormer is a commercially available "flyback" television transformer (79E77-15AC) which can be purchased for about $3. The voltage induced in the secondary is about 3000 V which produces a spark between the stylus and the table A_2 to give a black dot on the teledeltos paper. The maximum counting rate of the circuit is 300 c.p.s.
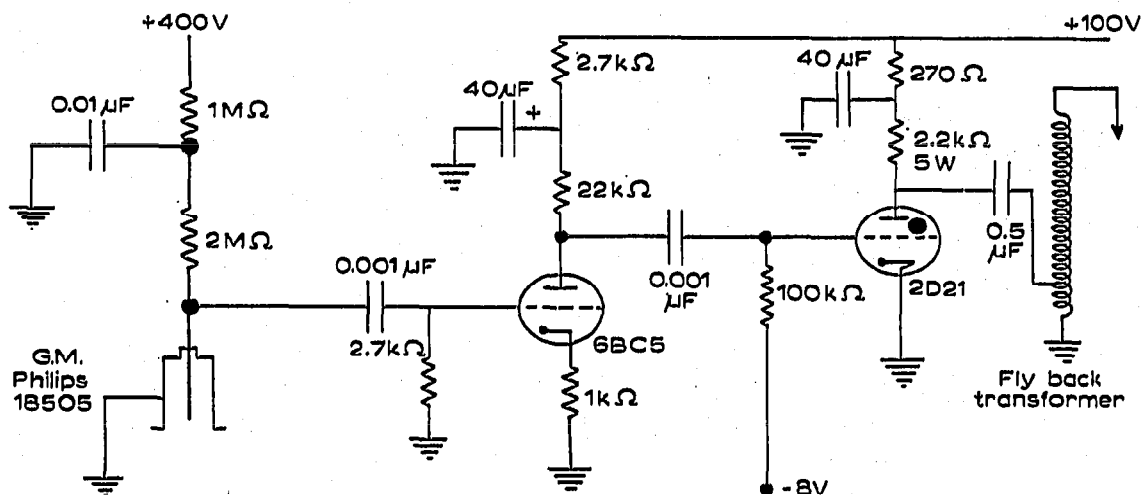

Fig. 3. Circuit diagram for converting G.M. pulses into high voltage pulses to produce sparks between the stylus and table A_2.

The speed and direction of the X motor is controlled by a circuit described elsewhere[5]. In this control the field coil of the motor is excited by a.c. while the armature carries pulsed d.c either in phase or out of phase with the field current. The portion of the half cycle in which current flows is determined by the setting of a potentiometer. The half cycle in which the current flows is determined by the forward-reverse switch.

The spacing of the scan lines is determined by a time circuit[6] which turns on the Y space motor for an adjustable length of time at each end of a scan.

Procedure for locating the streaks on the electropherogram

After a scan is completed, a correlation between the dot pattern on the teledeltos paper and the radioactive streaks on the electropherogram is made in the following way. An aluminum bar (Fig. 1 bottom) which contains two pins P_1 and P_2 is fitted into holes O_1 and O_2 on table A_2. Pencil marks are made on the teledeltos paper through two holes Q_1 and Q_2 in the bar. The teledeltos paper is then superimposed on the wet electropherogram sitting on table A_1. The aluminum bar is now placed so that the two pins P_1 and P_2 fit into holes X_1 and X_2 on table A_1, and the teledeltos paper is moved until the two holes Q_1 and Q_2 on the aluminum bar match the pencil marks thus exactly aligning the teledeltos paper with the electropherogram. Impression marks are then made around the regions of the teledeltos scan which are of interest and these marks then appear on the electropherogram. Regions of interest can then be cut out and the photoproducts eluted for further study, or counted in a scintillation counter.
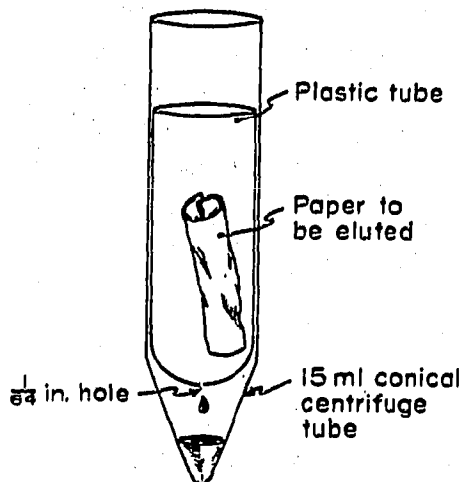

Fig. 4. Arrangement for quickly eluting compounds from paper strips cut out from wet electropherograms.

Elution technique

Fig. 4 illustrates the arrangement for carrying out a quick elution of materials from wet paper strips cut from an electropherogram. The paper strip containing the radioactive material is placed in a plastic tube which has a 0.04 cm hole in its bottom. The plastic tube is then inserted in a 15 ml conical centrifuge tube (see Fig. 4) and centrifugation carried out for one minute at 1000 r.p.m. About 85 % of the radioactive material is eluted off the paper in this manner. Increasing amounts of the remaining 15 % can be eluted by adding water or buffer solution to the paper strip and repeating the procedure.

RESULTS OF TYPICAL SCANS AND DISCUSSION

Figs. 5b–e illustrate some of the results of typical dot scans of a mock electropherogram. The mock electropherogram was prepared as follows. Paper strips 2.5 cm
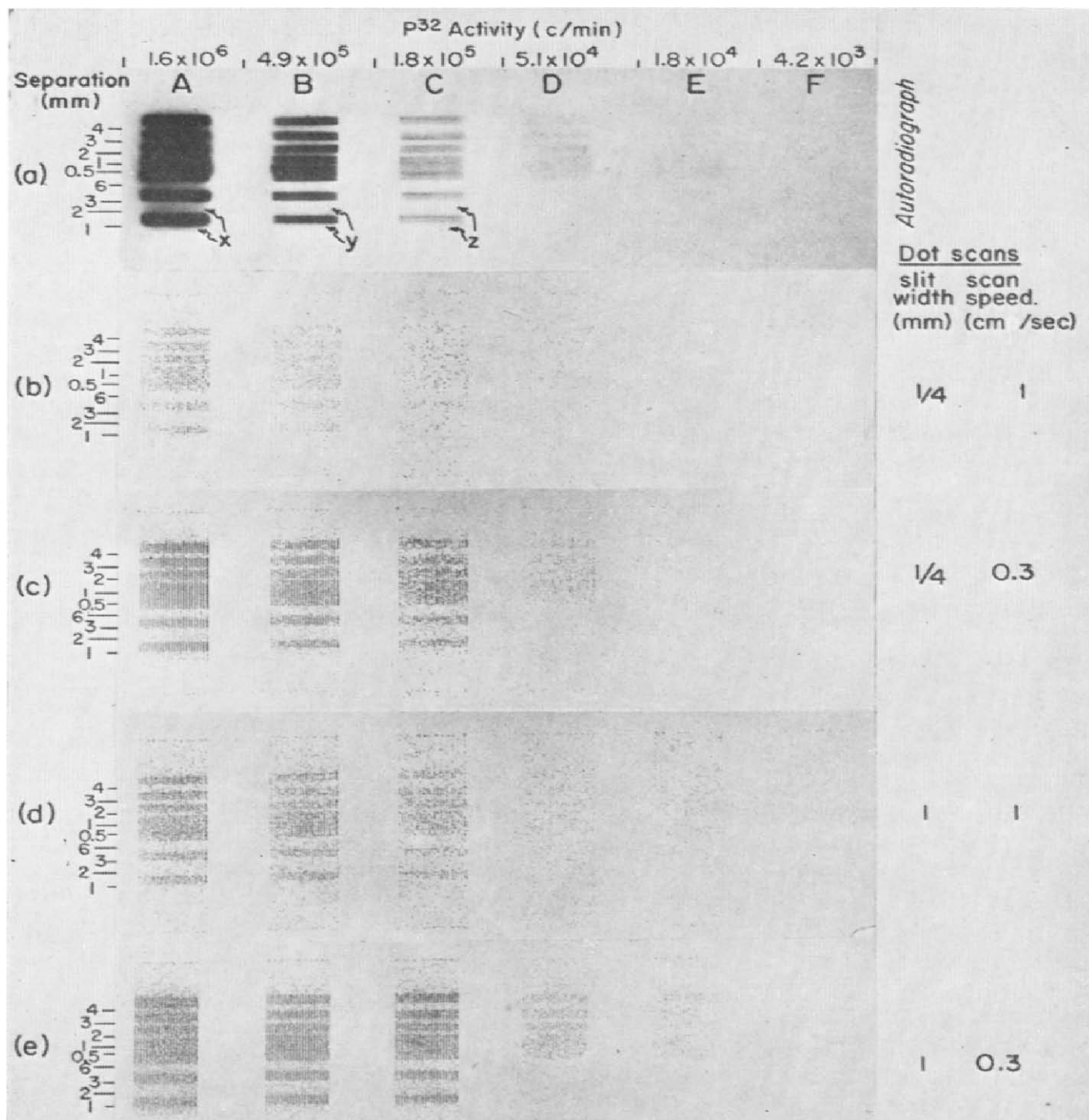
Fig. 5. (a) Autoradiograph of a mock electropherogram with the activities given at the top and the spacings shown on the left. (b), (c), (d) and (e) Dot scans of the electropherogram. x, y and z are paper strips containing 3% of the adjacent activities. The dot scans are shown for two typical cases of collimator slit widths and scan speeds shown on the right.

long and 0.3 cm wide were impregnated with different amounts of activity by soaking the paper in different concentrations of ^{32}P solution. These paper strips were then dried and fixed on a sheet of cardboard with the spacings and activities indicated in Fig. 5. Fig. 5a is an autoradiograph of this mock electropherogram made by exposing

it to an X-ray film (Kodak type KK). The corresponding scans of this electrophero-gram taken under a variety of conditions are shown in Fig. 5b–e. The scan speeds and collimator slit widths are indicated on the right hand side of the figure. In order to aid in interpretation of the scans we have indicated on the left side of each scan the separations of the strips given in Fig. 5a. An examination of Fig. 5 leads us to the following conclusions.

(1) For the highest activities (column A), Fig. 5c gives the best scan suggesting that the optimum conditions involve narrow slits and slow scans. However when the available activity is much less as in column D, then Fig. 5e gives the best scan. For this scan a wider slit (1 mm) and a slow scan was used. If very large activities were involved, a rapid scan and narrow slit would be advantageous but no example of this situation is given in Fig. 5. In general then the optimum conditions of scanning will depend upon the activity being located.

(2) A 2 mm separation between *equal* activities can easily be resolved for either slow or fast scans with slit widths up to 1 mm for activities given in A, B, C and D.

(3) Activity which is only 3 % of the adjacent activity and separated from it by 3, 2 or 1 mm cannot be resolved. That this is so can be seen in columns, A, B and C where activities X, Y and Z which were 3 % of the adjacent activity were impossible to observe. In other experiments we found that activities which were 10 % of the adjacent activity could be located if the separation was 2 mm or more.

(4) For very low activities it is sometimes useful to increase the slit width to 3 mm, but this destroys almost all the resolution. An activity of 4.2×10^3 c.p.m. on a strip 3 mm $\times$ 25 mm $=$ 75 mm^2 can just be seen (column F, Fig. 5e) and can easily be seen with a 3 mm slit (not shown). Since the effective lenth of the slit is about 15 mm the area of the aperture at 3 mm slit width is about $3 \times 15 = 45$ mm^2. The activity being looked at, at one instant, is about $(45/75) \times 4.2 \times 10^3$ c.p.m. $= 2.4 \times 10^3$ c.p.m. Thus the limiting detectable concentration is $(2.4 \times 10^3)/75 = 30$ c.p.m./mm^2. The scanner could detect lower activity by using counters with very low backgrounds and very slow scanning speeds.

The total time involved to obtain a dry electropherogram at room temperature, expose the fastest X-ray film at room temperature (Kodak type KK), and dry the autoradiograph is at least 2 h. The same information can be obtained from a wet electropherogram in 5 min using our scanner all the while maintaining the products at 0°. This comparison clearly indicates the usefulness of the device in dealing with unstable photoproducts.

It must, however, be stressed at this point that the real advantage of using this machine lies in providing a quick method of locating unstable radioactively labelled compounds immediately after an electrophoretic separation (the paper being wet) and for preparing stock solutions of these unstable compounds for further kinetic studies. For stable compounds containing [32P] labelled activity of less than 30 d.p.m./mm^2, the autoradiographic technique is more sensitive and useful than the scanner.

The scanner has been employed to isolate and study the photoproducts of [32P]CpC[7,8]. It has been particularly useful for locating unstable photoproducts with half lives as short as 1 h. It would also be useful in detecting photoproducts labelled with [14C] but could not be used to detect [3H] without major modifications involving the use of a windowless counter.

SUMMARY

The design, construction and operation of a scanner to rapidly locate radioactive streaks on an electropherogram is described. Using this scanner and a rapid elution technique one can isolate unstable compounds from wet electropherograms in about 6 min while maintaining the products at $0°$. The performance and limitations of the scanner are described. It will detect about 30 disintegrations per minute of ^{32}P activity per mm^2 and will resolve radioactive spots separated by as little as 2 mm. Using the scanner we have been able to successfully isolate a number of unstable photoproducts from [^{32}P]CpC and study their properties. The scanner can also detect photoproducts containing a ^{14}C label, but detection of ^{3}H will involve modifications involving use of a windowless counter.

REFERENCES

1 H. E. JOHNS, J. C. LeBLANC AND K. B. FREEMAN, *J. Mol. Biol.*, 13 (1965) 849.
2 K. B. FREEMAN, P. V. HARIHARAN AND H. E. JOHNS, *J. Mol. Biol.*, 13 (1965) 833.
3 S. ARNOFF, *Nucleonics*, 14, No. 6 (1956) 92.
4 C. W. GILBERT AND J. P. KEENE, *Radioisotopes in Scientific Research, Proc. UNESCO Intern. Conf. Paris*, 1 (1957) 698.
5 *G. E. SCR Manual*, 3rd Ed., General Electric Co., 1964, p. 144.
6 *G. E. Transistor Manual*, 7th Ed., General Electric Co., 1964, p. 321.
7 P. V. HARIHARAN AND H. E. JOHNS, *Photochem. Photobiol.*, (1967) in press.
8 P. V. HARIHARAN AND H. E. JOHNS, *J. Mol. Biol.*, (1967) in press.