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# USE OF THE SPARK-CHAMBER RADIO-SCANNER FOR RAPID LOCATION AND QUANTITATION OF LABELLED COMPOUNDS AFTER ONE- AND TWO-WAY SEPARATIONS

IVOR SMITH*, SUSAN E. MARCH, P. E. MULLEN and P. D. MITCHELL
*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W1P 5PR (Great Britain)*

## SUMMARY

A rapid method of scanning two-way radio-chromatographic and electrophoretic separations is described. The scanner produces a photograph in approximately 10 min, which is adequate for almost all uses and compares well with the usual 4- to 20-week X-ray film method. The technique is illustrated with examples from tyramine metabolism.

## INTRODUCTION

The need to identify the components of often complex mixtures is basic to the chemical and biological sciences. Although a number of substances have characteristic reactions which serve to identify them, such as spot tests for metal ions or immunological reactions for proteins, it is generally more accurate to carry out these tests after one or more preliminary separations have been performed on the original mixture. Chromatographic and electrophoretic separations on paper or thin layers are, in principle, ideal and, consequently, have given rise to a variety of techniques and a range of commercially available technology. The techniques are extremely simple and quick to perform, and they are relatively inexpensive. The separated substances are located within the finite area of paper or layer used (compare column separations) and one or more of them can readily be recovered in solution. Finally, and a point not often realised, the separation gives comparable results on the micro-, semimicro- and macro-scales, for it is quite possible to separate tens of milligrams of material on a single sheet or layer.

Although paper chromatography (PC) and thin-layer chromatography (TLC) have come into wide general use, the corresponding electrophoresis techniques are comparatively rarely used outside the fingerprinting world of macromolecular sequence studies. This is regrettable as high-voltage separation techniques are often simpler and quicker to perform than the corresponding chromatographic separations. Moreover, being based on a different principle, electrophoresis can often bring about

* To whom requests for reprints should be sent.

separations which are difficult by chromatography. The scanner to be described is equally applicable to both methods.

The first spark-chamber scanner was described by Pullan and Perry[1] in 1965, but the present type is a modification designed by Pullan[2] in 1968. It has been described in detail by Hesselbo[3] and only a general description will be discussed here.

## DESCRIPTION OF THE SCANNER

In principle the spark-chamber consists of a set of coiled copper cathodes connected in parallel, each with a central stainless-steel wire anode, these also connected in parallel (Fig. 1). About 50–60 coils, each approximately 25 cm long, are cemented to a plate of glass such that the overall useful area of the plate is 25 $\times$ 25 cm. Hence the usual 20-cm-square TLC plate or 25-cm-square paper can be examined. Larger papers must be scanned more than once for a full coverage. Each cathode plus central anode constitutes an independent condenser and when any short is applied across the system a current will flow. As used here, a radio-disintegration will occur at the position of any one of the separated radioactive substances. This will be evidenced by a spark jumping the electrode gap and these sparks can be photographed to form a picture of the two-dimensional separation. The gap between the electrode plate and the chromatogram lying on the base of the apparatus is a few millimetres and the space is flushed with 10% methane in argon for some moments before use. Photography is by means of a Polaroid camera fixed in position at the top of a black chimney place above the electrode plate (Fig. 2).

The apparatus is suitable for use with any isotope, functioning extremely well with $^{14}C$ and $^{32}P$ and less well, but quite adequately, with tritium. Photographs can usually be obtained within 10 min but exposures of up to 1 h can yield good pictures as can such short exposures of 5 sec with very hot plates.

In order to locate the accurate position of the separated spots, on the original paper or layer, two tricks are used. Firstly, a radioactive grid is included with every chromatogram scanned to enable the picture to be re-aligned the right way up with the original. The grid is a series of radioactive ink dots in two dimensions, one set of dots running along the origin line and two sets of differently spaced dots running in

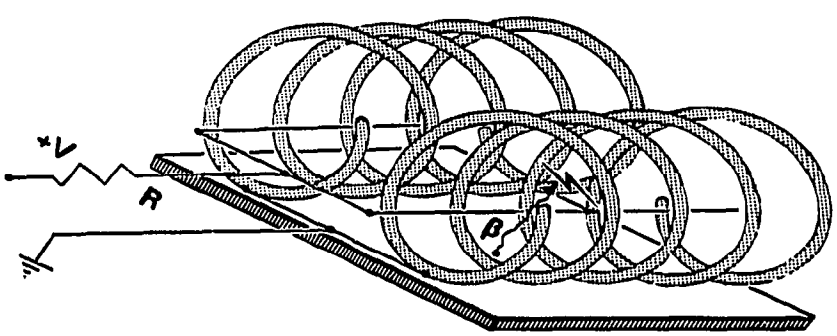



Fig. 1. Diagram showing two cathode coils linked in parallel and their respective anode wires running centrally and also linked in parallel. The $\beta$ represents a radio-disintegration with emission of a $\beta$ particle which discharges across one anode–cathode pair with the liberation of a visible spark.

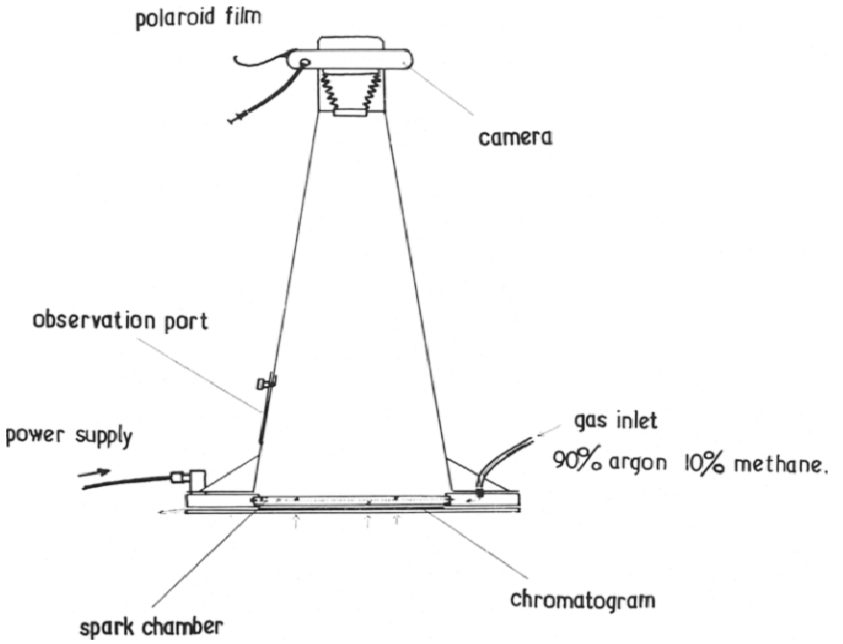



Fig. 2. Diagram of the complete spark-chamber set-up showing the spark-chamber plate inverted over the chromatogram and the vertical chimneyhood with Polaroid camera at its apex. The hood has an observation port to check that sparks are forming, but we also have an amplifier which picks up the sparking noise indicating the apparatus is functional.

the solvent flow direction. The grid and chromatogram are always placed in the apparatus in a standard way so that mixed re-aligning cannot occur.

Secondly, the small Polaroid picture is placed, face upwards, and covered with a sheet of glass or plastic film, and the spots are carefully ringed. This sheet is then placed on an overhead projector, beamed onto a wall on which the chromatogram is pinned, and enlarged until the grid spots from the beam become exactly co-

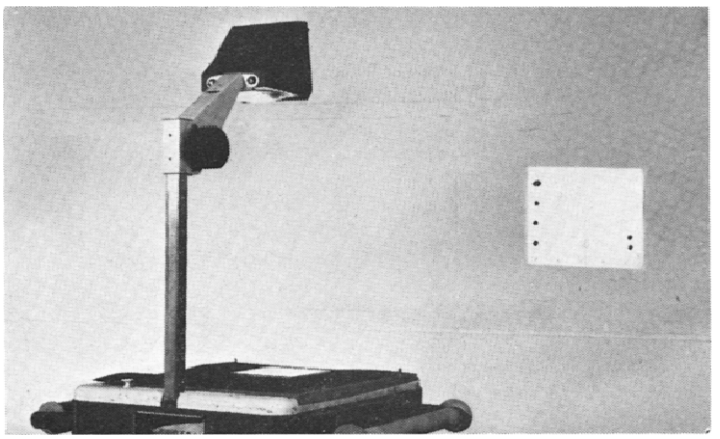


Fig. 3. Overhead projector scheme for enlarging the Polaroid picture back onto the original chromatogram. The chromatogram is surrounded by a mask so that only that light passing through the picture is beamed onto the plate as this simplifies the task of aligning the grid spots.

incident with those of the original grid. Then the other spots from the separated compounds must also be coincident with their beamed spots, so these can be carefully encircled to mark their positions permanently. Substances with very similar mobilities can be readily delineated in this way (Fig. 3).

It was originally hoped that quantitative data would also be obtained via extensive computer hookup. Unfortunately, this is not possible. Very hot spots act as spark sinks such that hot spots appear even hotter, and less active spots appear weaker than they really are. Nevertheless, quantitation is readily achieved by cutting out the respective areas of paper, placing these in one of the usual POPOP mixtures and counting in a liquid scintillation counter; differently sized pieces of paper have practically no effect on counting background, etc. In one sense, therefore, the visual picture can be misleading and it must always be remembered that the picture observed is entirely a qualitative one within very wide limits.

## APPLICATIONS

The authors have been interested in the metabolism of tyramine (T) in man for some time now and, in what follows, they will illustrate the uses of the spark chamber by applications in this field. All the photographs shown are 5- to 15-min exposures in the scanner. The diet contains many aromatic compounds and many dietary compounds even contain tyramine, *e.g.* cheese. Hence the only reasonable way to study this compound is by means of a radioactive label and our normal procedure is to feed 100 mg tyramine together with 50 $\mu$Ci $^{14}$C-labelled tyramine ($110 \times 10^6$ d.p.m.). 95% of the total radioactivity is recovered in a 9-h period in a volume of 500–1000 ml urine. 250 $\mu$l urine, *i.e.* approximately 25,000 d.p.m., is used for separation. The scanner will pick up 500 d.p.m. in a 10-min exposure quite easily, showing that metabolites representing as little as 1% of the administered dose will be readily observed.

A one-way separation of 250 $\mu$l urine shows four main bands of radioactivity.

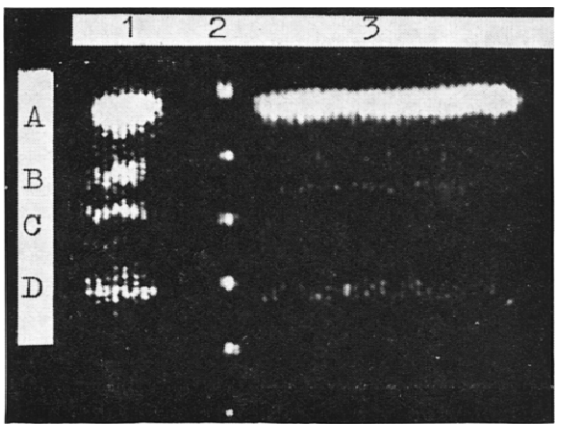
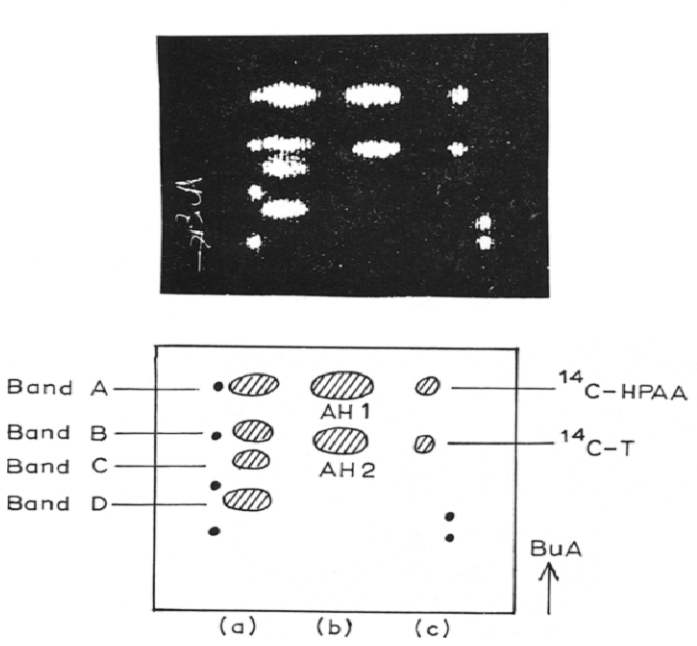



Fig. 4. One way-separation of tyramine metabolites after 10-min exposure. Four bands (A, B, C and D) of radioactive material are separated. Column 1 shows separation after 250 $\mu$l are applied, column 2 shows grid spots and column 3 a streak separation of 2 ml urine for recovery of substances prior to further analysis.

Bands A and D are, respectively, *p*-hydroxyphenylacetic acid (HPAA) and tyramine-O-sulphate, whereas bands B and C are mixtures (Fig. 4). The quantitative analysis following scintillation counting shows Band A = 68–80%, B = 2–7.5%, C = 3.7–6.7% and D = 9–19%, which indicates a reasonably expected normal variation together with a reasonable reproducibility for the method although, of course, individual specimens give identical results on triplicate analyses. The same urine, after total acid hydrolysis, yields two bands, namely HPAA and T (Fig. 5). Ether extraction[4] removes only the HPAA, which, on hydrolysis, is confirmed as being a single substance. On drying the ether-extracted aqueous residue and extracting with chloroform–methanol (1:1)[4], spots corresponding to bands B, C, and D are observed and, on hydrolysis, these yield the same two bands of HPAA and T previously found (Fig. 6). However, on counting the isolated bands, band D always corresponds to the lower band found after hydrolysis, showing that this lower band is T and that band D is the only conjugate of T. Hence bands B and C must be conjugates of HPAA and this is also confirmed by showing that the counts in bands B and C correspond exactly to the counts in the upper HPAA band found after hydrolysis. These experiments require less than a week to perform because of the instant picture of radioactivity obtained after each experiment.

Another set of examples is the comparison by two-way separations of the



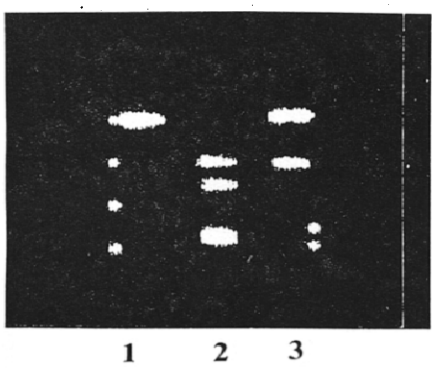




Fig. 5. Chromatogram of urine run in butanol–acetic acid–water (60:15:25) (BuA) (a), urine after total acid hydrolysis (b), and a standard mixture of T and HPAA (c). Careful examination shows that band B is not identical with AH 2, which is T. This is readily confirmed by chromatography in a second solvent and high-voltage electrophoresis at pH 5.3.

Fig. 6. Original urine extracted with ether (column 1) showing only Band A; HPAA is extracted. The residue from the ether extract is treated with chloroform–methanol (1:1), which dissolves bands B, C, and D and chromatographed before (column 2) and after (column 3) acid hydrolysis.

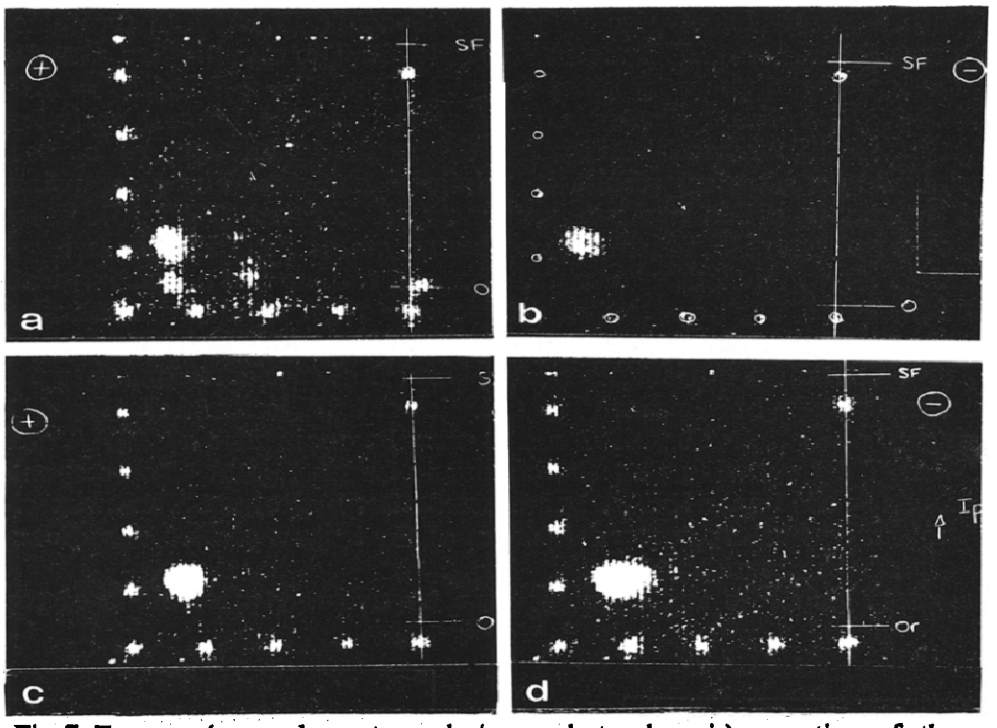



Fig. 7. Two-way (paper chromatography/paper electrophoresis) separations of ether extracts of urine from the rat (a), the rabbit (b and c) at different concentrations, and man (d) after an oral dose of T. Solvent: isopropanol–water–880 ammonia (8:1:1). Note the more elaborate system of grid spots in this and the next figure.

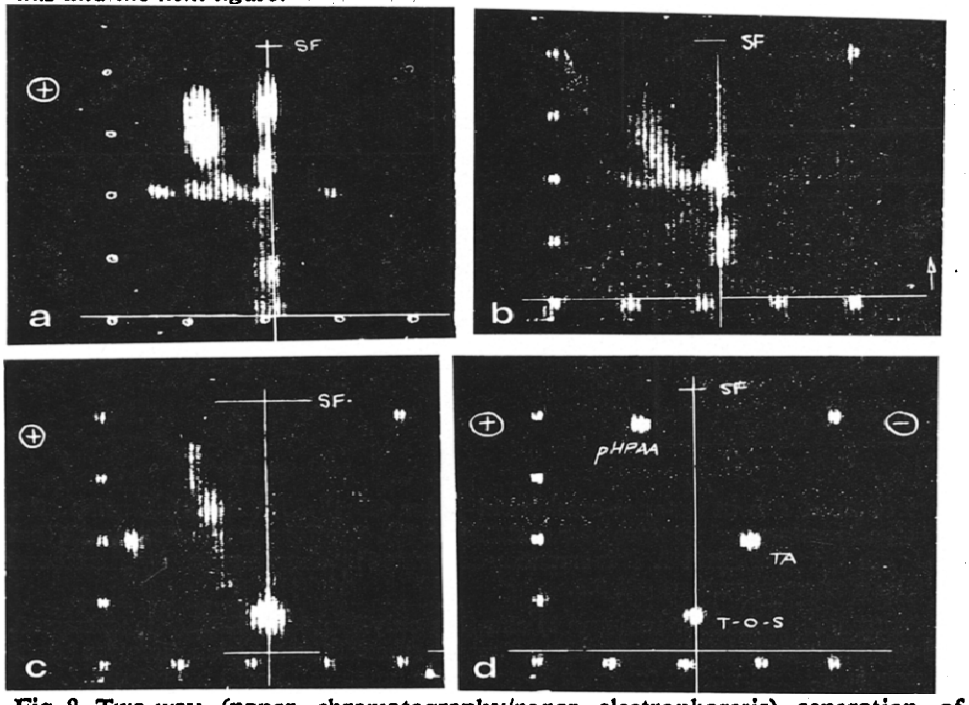



Fig. 8. Two-way (paper chromatography/paper electrophoresis) separation of a chloroform–methanol (1:1) extract of the residues of ether extracts of urine of the rat (a), the rabbit (b), and man (c), together with a set of standards (d). Solvent: butanol–acetic acid–water (60:15:25).

metabolites of T in man, the rabbit and the rat. Ether extracts in man and the rabbit both yield only HPAA, whereas the rat shows a number of metabolites to be present (Fig. 7). Chloroform–methanol extraction of the residue shows that each species forms a number of different metabolites (Fig. 8) and these experiments are useful for seeking animal models for human metabolism.

The authors were particularly interested to determine whether free T was present in any of the urines, as T is known to be present in all urine. However, using a high-voltage electrophoresis technique designed specifically to separate basic compounds, they were unable to find any free T at all following oral administration to man (Fig. 9), although they readily observed it on running standard T and after intravenous administration. This suggests to them that urinary T is endogenous in origin.

One example of tritium-labelled compounds has been examined at the request

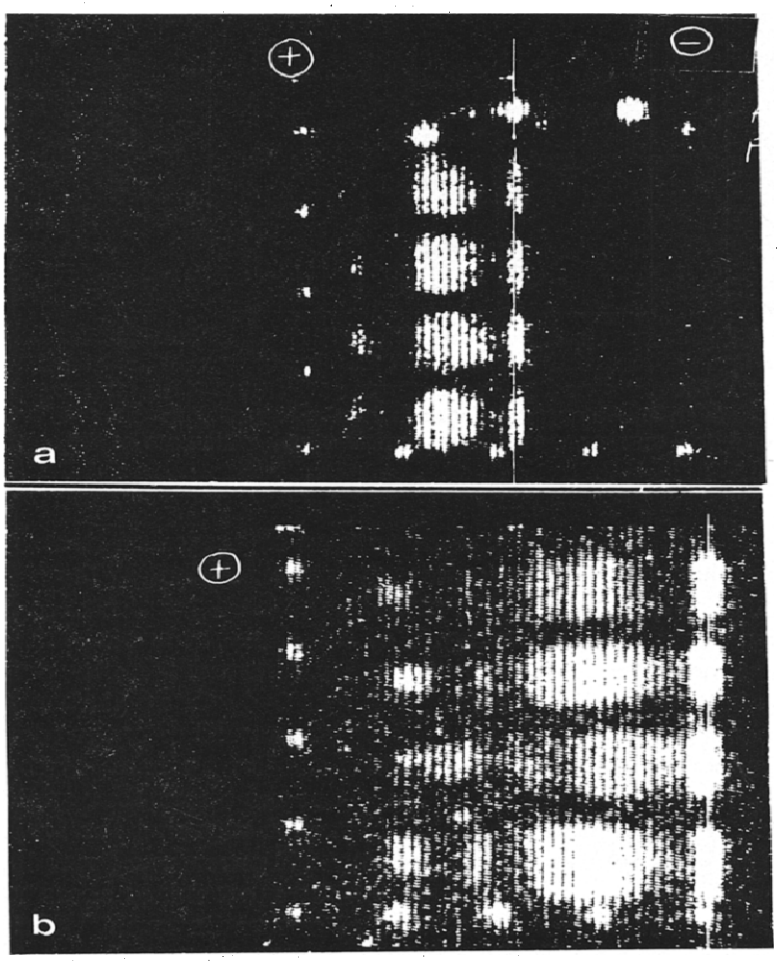



Fig. 9. One-way high-voltage electrophoresis of tyramine metabolites at pH 5.3. The central origins in (a) with the T standard show that no free radioactive tyramine is present. The origin in (b) is at the far right in order to effect a maximum separation of neutral and acidic metabolites across the whole length of the 10-in. paper.

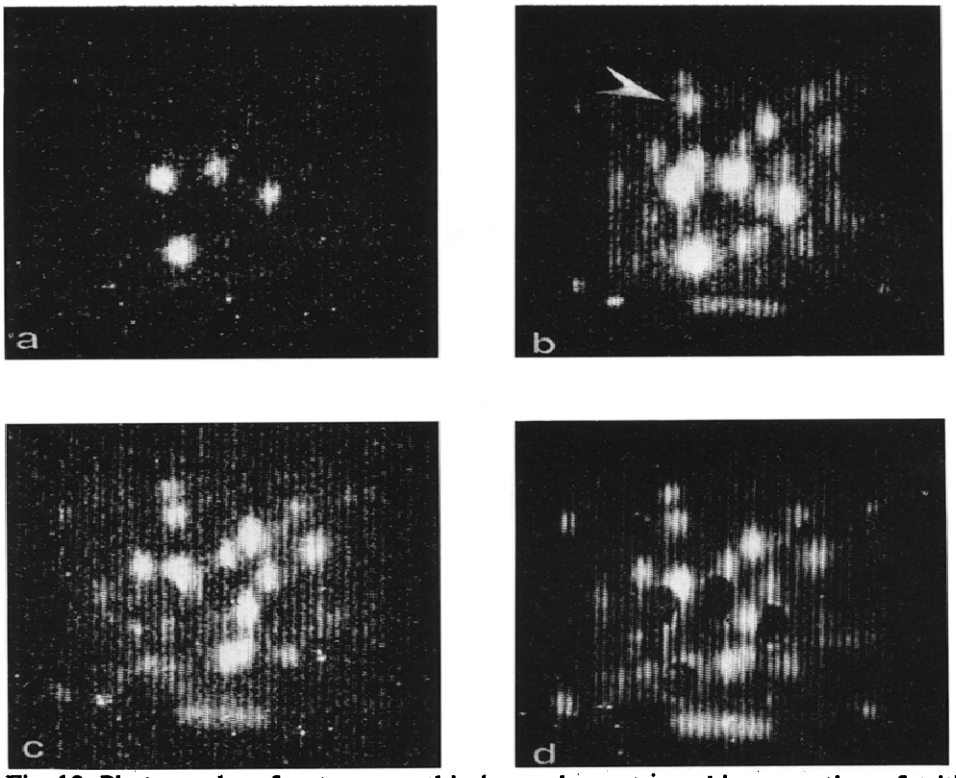



Fig. 10. Photographs of a two-way thin-layer chromatographic separation of tritium-labelled nucleotides obtained from an enzymic digest of a purified tRNA. (a) A 15-min exposure at f22 using Polaroid Land film Type 107 in a Model 180 camera; (b) 1-h exposure at f32; (c) a 1.5-h exposure at f32 with the four major spots masked; (d) a 3.75-h exposure at f64 with the four major spots masked. Figs. b, c, and d show four peripheral grid marker spots and a marker streak which are at the sides of the 20 × 20 cm plate.

of Dr. Randerath[5], who provided the thin-layer chromatogram of separated nucleotides. As the tritium decay particle energy is approximately one tenth that of the $^{14}C$ particle energy, the Melinex sheet covering the spark chamber had to be removed and the exposure time increased appreciably. Radioactivity of the different spots varied over a factor of about sixty times, the lowest (detected by autoradiography and indicated by an arrow in Fig. 10b) being 0.3% of the total of 3–4 $\mu$Ci applied. Exposure of the whole chromatogram for 15 min at f22 aperture showed only the four major spots (Fig. 10a), whereas exposure for 60 min at the smaller aperture of f32 gave the results shown in (Fig. 10b). However, on masking out the above four spots by six thicknesses of Whatman No. 1 paper (1 mm thick), and re-exposing for 1.5 h with an aperture of f32 Fig. 10c was obtained. The masking-off procedure prevents the major spots from acting as electron sinks, thus allowing the most minor spots to be visualised, including five spots not seen on the original autoradiograph, which was obtained after a four-day exposure. Hence, within a much smaller time it is possible to take a number of photographs with the spark chamber, to mask off and/or excise the major radioactive spots and to locate specifically the quantitatively minor spots or compounds present.

These examples illustrate the wide applicability of the spark-chamber scanner and the tremendous time-saving possible, when compared to the usual X-ray film method of location of radioactive compounds.

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