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DETECTION OF TRITIUM ON PAPER CHROMATOGRAMS*

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SUMMARY

A technique is described whereby ^3H compounds may be detected on paper chromatograms. The technique involves soaking the chromatogram in a liquid scintillator so that the energy of the disintegrating tritium atoms is converted into light which is detected by a film. Thus a weak β-particle is converted into light quanta which can travel into the film to produce an image. By the use of fast film, small amounts of ^3H compounds can be separated and identified in only a few days.

INTRODUCTION

Hydrogen is an element of fundamental importance in biological processes. It is therefore important to have a method for studying its pathway through living systems. Paper chromatography[2] is an extremely powerful technique for the separation of complicated mixtures of compounds and has found much application in the field of biochemistry. One of the greatest problems of paper chromatography is the detection of the individual compounds on the paper. This has been done by spraying the paper with suitable chemicals which develop colour reactions with the compounds

* A brief account of this technique has been published[1].
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of interest. Unfortunately this usually destroys the compounds and is not of general application. In the case of radioactive materials a film may be laid on the chromatogram[3] which on development gives dark areas corresponding to the radioactive compounds. This technique[3] works well for ^{14}C, and ^{32}P, but in the case of ^{3}H the maximum energy of the β-particle (18 KeV) is too low for it to leave the paper and enter the emulsion of the film. This paper describes a technique whereby this difficulty is overcome.

DESCRIPTION OF METHOD

The technique involves soaking the chromatogram in a liquid scintillator so that the energy of the disintegrating tritium atoms is converted into light quanta which can travel into the film to produce an image.

In actual practice the chromatogram is attached, with wire staples, to some convenient backing material (used X-ray film is suitable). Marker segments, which are pieces of filter paper on which have been placed tritium labelled compounds (see

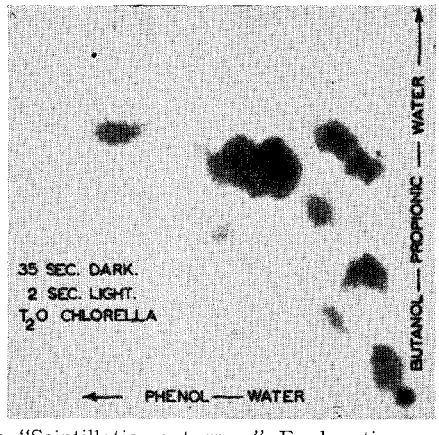

Fig. 1. "Scintillation autogram". Explanation see text.

below for more details), are attached to the corners of the backing sheet with staples. These later provide reference points on the backing sheet and also on the film with which to line up the film and the original chromatogram, thus identifying a spot with a definite area on the paper chromatogram. The chromatogram sheet thus prepared is laid in the bottom of a shallow tank and covered with a scintillating liquid (see below for discussion of scintillation liquids). A sheet of screen type X-ray film (see below for discussion of film) is next placed on top of the chromatogram, taking care to eliminate any bubbles. The tank is then covered with an air-tight top. After a few days, the film is removed from the tank, drained of scintillating liquid and allowed to dry in the air of the dark room. When all the toluene has drained or evaporated from the film, it is placed in the wash water and wiped with a soft cloth to remove any terphenyl which may have remained. The film is then developed and fixed in the normal manner. Such a "scintillation autogram" is shown in Fig. 1. Thus, as in ^{14}C radioautography, compounds can be tentatively identified by their position and this identification confirmed by removal from the paper and co-chromatography with known compounds, or by chemical treatment and re-chromatography.

DETAILS OF METHOD

Apparatus

The author's tanks are made by soldering 1/4" square extruded brass rod onto a heavy gauge brass sheet of suitable size taking care to keep the top as flat as possible. Another piece of brass plate serves as a lid which is kept in place with a lead brick. The tanks are kept in a dark-room cupboard.

Scintillation liquid

The classical scintillation solution viz: 3 g/l *p*-diphenyl benzene (terphenyl) in toluene which is used for scintillation counting is quite satisfactory. The presence of sulphur compounds in toluene may cause some quenching of the light but this is a relatively small effect and can be overcome by the use of "sulphur free" toluene which is commercially available, or by passing the toluene down an alumina column. Since commercial medical "screen type" X-ray film is made to be used with a calcium tungstate fluorescent screen the sensitivity of the method can be improved some 40 % by adding a suitable "wavelength" shifter (*e.g.* diphenyl heaxtriene 0.01 g/l) to the scintillating solution. This makes the fluorescent spectrum of the solution match more closely the wavelength response of the film.

Marker segments

Marker segments are small pieces of filter paper which have been impregnated with some tritium labelled compound. They are attached to the corners of the backing sheet along with the chromatogram and serve as markers which enables the developed film to be lined up with the chromatogram for spot location. They are prepared by placing a spot of labelled tritium compound, (*e.g.* algae or yeast which has been treated with tritiated water) on a piece of filter paper which, after drying, is cut into segments.

Film

For maximum sensitivity it is advantageous to have as fast a film as possible. For practical purposes it is also an advantage to have the film in reasonably large sheets. The manufacturers of film for normal photographic purposes sacrifice speed (even in the very fast films) in order to keep the grain size down. Screen type X-ray film on the other hand is much faster to light than even the fastest of normal photographic films. That it has a large grain size is not important in this application. Screen type X-ray film (such as Kodak "Blue Band") has the added advantage that it is readily available in large (14" × 17") sheets which are used for conventional chest x-rays. It also can be used with a safety light since it is relatively insensitive to light of the longer wavelengths.

Film processing

The developer and fixer as supplied by the film manufacturers when used according to their directions will produce the optimum image. Care should be taken, however, to dip the film in water and wipe it with a soft wet cloth to remove any terphenyl before placing in the developer.

Exposure time and application to ^{14}C chromatograms

A question that immediately presents itself is whether this technique can be applied to very low level ^{14}C chromatograms which take a very long time to expose in the normal manner. Unfortunately this technique is of no help in this problem. The technique is more sensitive for short exposures (< 24 h) than is conventional radioautography, but for longer exposures it is progressively less sensitive. This is in the nature of the photographic process itself. In conventional radioautography one is developing the actual β-particle tracks and the intensity of these is to a first approximation independent of the exposure time. In the case of the photography of light, however, which is the principle of the scintillation autography technique, there exists for a given film an optimum time during which a given quantity of light will produce the greatest effect. This period is much less than a day for any film. Thus for longer exposures the effect of a given quantity of light decreases as the length of time over which it is received increases. Thus, if long exposures (many weeks) are necessary for weak ^{14}C chromatograms then conventional radioautography is the best approach. Similarly for tritium experiments enough isotope should be used to get the desired exposure in less than a week.

Sensitivity

This of course depends on the length of exposure time, but if 3 g/l terphenyl and 0.01 g/l diphenyl hexatriene in toluene are used with Kodak "Blue Band" film then a spot whose area is one square cm, and whose activity is 0.1 μC can be easily detected after an exposure of 50 h.

DISCUSSION

An example of a scintillation autograph is shown in Fig. 1. As can be seen the quality and sharpness compares favourably with radioautograms produced by the β-particles from ^{14}C. Because of the universal occurrence of hydrogen in biological systems, and the availability, low health hazard and cheapness of tritium (10^{-4} that of ^{14}C) coupled with the ease of preparation of ^{3}H compounds, this technique should find wide application.

Tritium can be introduced easily into many organic molecules, either by hydrogenation of the corresponding unsaturated compound or by exposure to tritium gas[4]. This latter method of preparation (The Wilzbach synthesis) seems to be of a very general application, but suffers from the fact that it yields a product contaminated with small amounts of ^{3}H material of high specific activity. With the technique described in this paper, however, it is possible to prepare chromatographically pure ^{3}H compounds. CRAWFORD[5] has exposed a mixture of amino acids to tritium gas and separated the resulting mixture using paper chromatography and the above technique.

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VITAMIN D AND THE STRUCTURE OF KIDNEY MITOCHONDRIA*

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SUMMARY

An electron microscopic examination has been made of thin sections of kidney mitochondria isolated in 0.44 *M* sucrose from vitamin D deficient rats and from vitamin D treated rats. Vitamin D deficiency resulted in swollen and morphologically damaged mitochondria characterized by large intercristal spaces and disrupted cristal systems. These changes were prevented by vitamin D.

INTRODUCTION

The well-established accumulation of citrate in tissues following vitamin D administration[1] prompted our investigation of Krebs cycle oxidations in vitamin D treated and deficient rats[2]. It was found that vitamin D specifically reduced the oxidation of citrate and isocitrate by kidney homogenates and mitochondria[2,3]. This effect was also produced by the addition of vitamin D *in vitro*[4]. Further studies[5] to be published in detail later, suggested that the reduction in oxidation was not necessarily due to an enzymic inhibition, but was possibly due to physical inhibition of citrate penetration of the mitochondria. In support of this, it has now been found that the morphology of mitochondria isolated from vitamin D deficient rats differs markedly from that of vitamin D supplemented rats.

METHODS

Young, male Holtzman rats weighing 70–80 g were fed *ad libitum* an adequate Ca and P semisynthetic diet with and without vitamin D for 21–28 days as described earlier[1,2]. They were killed by a sharp blow on the head followed by decapitation. The kidneys

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