teflon pump with two independently adjustable cylinders, one pumping the gradient solvent to a magnetically stirred mixing vessel and the other pumping the resulting mixture into the column. After tube 100 the column temperature was allowed to rise to ambient room temperature (20°) which facilitated elution of DDA.

DDE was eluted with the starting solvent, then gradients were used for the remaining elutions as in Fig. 1. There was a clear separation of DDE, DDT, DDD and DDA. Although Kelthane and DBP were not completely separated here they should be resolvable by continuing the benzene gradient instead of using pure benzene elution. In the original metabolite work *o,p'*-DDT was present as a minor impurity and separated satisfactorily, eluting ahead of the *p,p'*-DDT in the position of the dotted line in Fig. 1. Recoveries in the present experiment ranged from 95% to 100% by gravimetry and these could no doubt be improved by more precise assay.

Silicic acid columns with various percentages of water in the adsorbent, a range of solvents, gradients, systems and temperatures gave a very flexible technique for separations and clean up of metabolic products, although reproducibility of results depended on standardisation of each batch of adsorbent. A table of systems utilised for this work (Table I), some of which were used in the production of the chromatogram represented in Fig. 1, gives some idea of the versatility which may be exploited for resolutions of organic solvent soluble compounds with very similar chromatographic properties.

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Permanent records of thin-layer chromatograms on transparent paper

Several methods are described in the literature for the documentation of thin-layer chromatograms[1,2]. A widely accepted approach is the spraying of the chromatograms with a polymer dispersion (Neatan, Brinkman) and the subsequent mounting of the plastic film on transparent acetate tape. Other methods are reported in which the chromatogram is photographed or copied as a blueprint[3–5] or reproduced on a sheet of transparent paper[1]. Advantages and disadvantages are inherent with each of these methods and in general the nature of the work in which thin-layer chromatography is used determines which kind of documentation will be most suitable.

During the course of preparative and analytical work on urinary constituents we found that the recording of chromatograms on transparent paper was the best way to process rapidly a large number of chromatograms without interference with further preparative or analytical procedures. This communication will describe our procedures

as they emerged out of several modifications and as they are now routinely used in our laboratory.

After the chromatogram has been marked under U.V. light or sprayed with the appropriate reagent it is covered with another uncoated glass plate of equal size. The adsorbent surface remains intact even if the glass plate is accidentally moved in relation to the chromatogram or when the covering glass plate is finally removed after a copy of the chromatogram has been made. A sheet of transparent paper (tracing paper) is then placed on top of this sandwich type pack and clamped to the glass plates by means of three or four clips normally used to fasten paper chromatograms to the supporting glass rods during development. The pattern of marked or colored spots can now be easily retraced on this sheet of paper. The spots can be numbered and, if necessary, pen or pencil notes can be written on the paper.

The ease with which the drawings of these copies can be done was considerably improved by the use of a light source shining through the chromatogram from underneath. For this purpose we used a light box slightly larger than the regular square thin-layer plate. An added advantage of the transmitted light was found to be in the enhanced detection of very weak spots which were not recognizable by inspection of the chromatogram under reflected light.

As a result of our experiences with this type of recording chromatographic patterns from thin-layer plates we designed a lightbox to further facilitate the use of

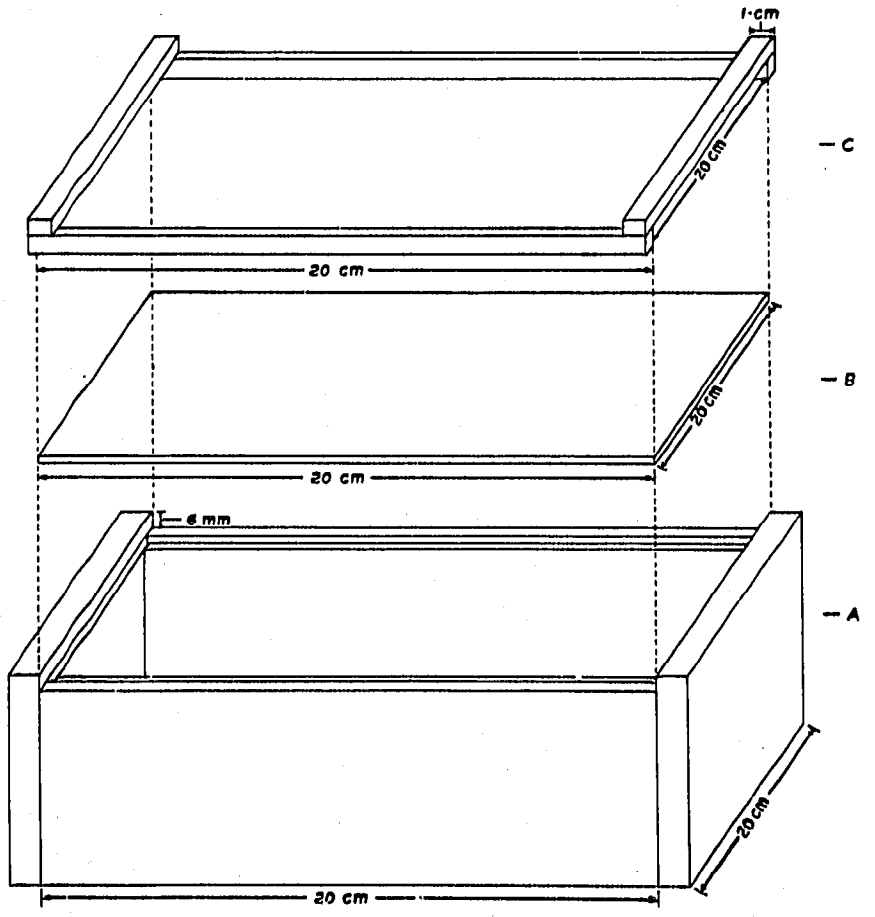

Fig. 1.

this procedure. Fig. 1 represents an exploded view of this device. It consists of a lightbox (A) as a base with slightly raised edges on both sides. The box should contain light sources with a low heat development (fluorescent light) to prevent the exposure of the plate to unduly high temperatures. The box is covered with a plate of frosted glass in order that the light be evenly distributed. The coated and developed thin-layer plate is placed on this glass plate and held in place by the raised edges of the box. A clean glass plate (B) is placed on top of the chromatogram followed by a sheet of transparent paper. Finally a frame (C) made from plastic material is placed on top of it all. This frame will keep the transparent sheet in position by bending it down in front and back. The 2 rims parallel to the raised edges of the light box will press the sheet flat to the glass plate so that a blurring of the contours is prevented. The measurements which are given in this sketch are only tentative because the measurements of thin-layer plates were found in our experience to vary slightly among the different manufacturers.

The advantages of transparent copies from thin-layer chromatograms were obvious in our work. This procedure will leave the surface of the adsorbent intact and will not interfere with further procedures. This aspect is important when the chromatogram is to be eluted subsequently for preparative or quantitative recovery of individual spots for analytical procedures. In this regard the use of the interposed glass plate was an important modification because we observed that a considerable amount of adsorbent tends to be removed from the plate when the sheet of paper is placed directly on the adsorbent layer and a close contact between sheet and adsorbent is established even under the slightest pressure of the drawing pencil. It is also important when multiple spray reagents are used and intermediate records are desirable. In addition, we observed that the absorbent layer of silica gel showed an occasional tendency to form bubbles and to flake off when certain solvent mixtures were used or after the plates were heated for the color development. An intact surface of the adsorbent layer, however, could be easily restored by compressing the raised bubbles as the protective glass plate was placed on the chromatogram for tracing.

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