A method for the more efficient utilization of thin-layer chromatoplates

A method has been devised for doubling the useful area of the Stahl thin-layer chromatoplates. The plates are coated with adsorbent in the usual manner and allowed to dry at room temperature for about 10 minutes. Then they are inverted and a layer of adsorbent is applied to the reverse side. Contrary to what might be expected such manipulation does not damage the underside. The doubly coated plates are cooked and stored as usual. However, care must be taken to prevent damage to the underside when the test material is spotted. For this purpose the plates may be placed on a frame which holds them slightly elevated from the work bench. The frame which touches the plate only along a thin outer border resembles a picture frame and is easily constructed from wood. The detection of the chromatographed material and the making of permanent records with a common letter copying machine are not hindered by the presence of adsorbent on both sides of a plate. This addition to the thin-layer chromatography technique proved very satisfactory while reducing the space and manipulations customarily required in thin-layer chromatography.

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Thin-layer chromatography of urinary metabolites of chlorpromazine and related psychotropic drugs

Thin-layer chromatography, in spite of manifold successful applications, is not currently applied to metabolic studies of psychotropic drugs.

In the course of further researches on the urinary excretion of chlorpromazine (CPZ) and chemically related compounds (chlorprotixene (CPX), imipramine (IP) and amitriptyline (ATL)), this technique has given better results than the techniques used in our previous, analogous investigations[1,2].

Thin-layer chromatography on Kieselgel-G layers was employed; chromatoplates (18 × 23 cm) were prepared and activated according to STAHL[3,4]. The most suitable solvent was the system *n*-butanol–acetic acid–water (88:5:7 for CPZ and CPX; 65:15:20 for IP and ATL). Ascending chromatographic runs of 12–13 cm were performed at room temperature (26–27° C), in closed vessels. The same procedure was carried out with normal rabbit urine to which the four compounds in appropriate concentrations had been added. Detection was performed by spraying the plates with concentrated H_2SO_4. CPZ and its metabolites exhibit spots of various colours, from red to violet; IP, ATL and related metabolites show green (IP) and brick-red (ATL) fluorescence under short wave U.V. light (250 mμ) after heating (95–100° C); CPX and its metabolites exhibit a clear orange-red fluorescence under Wood's light.

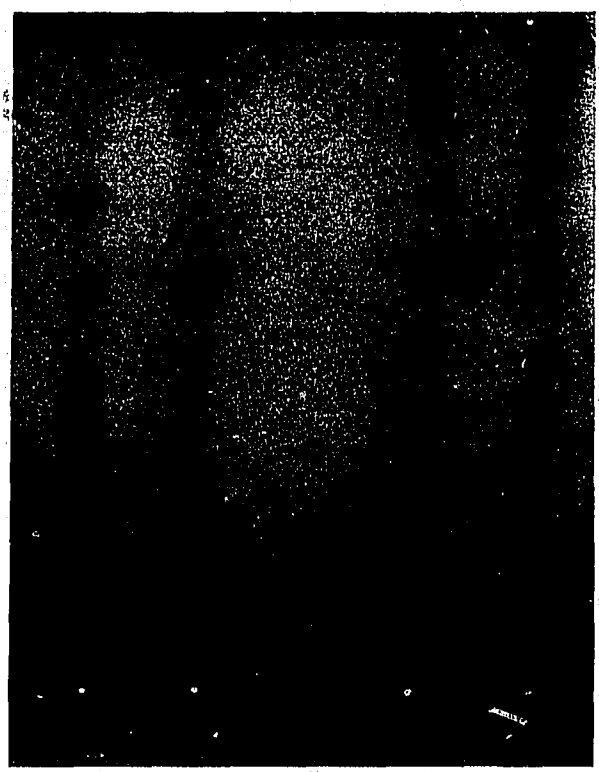

Fig. 1.

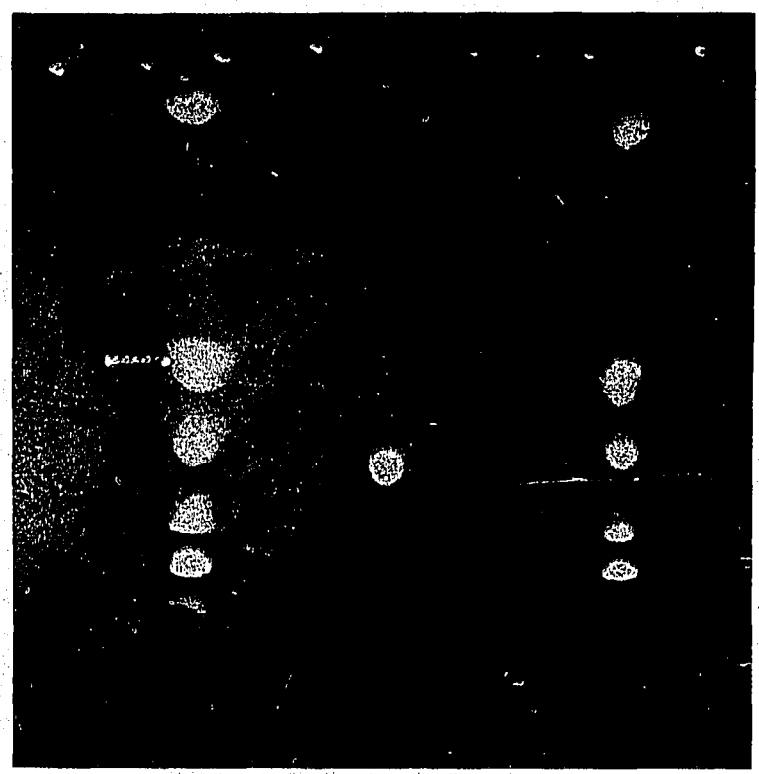

Fig. 2.

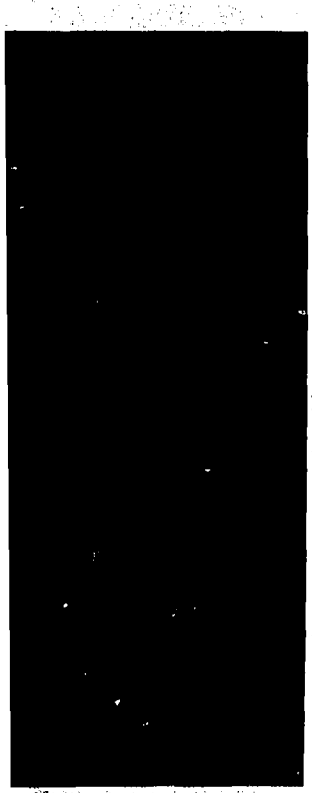

Fig. 3.

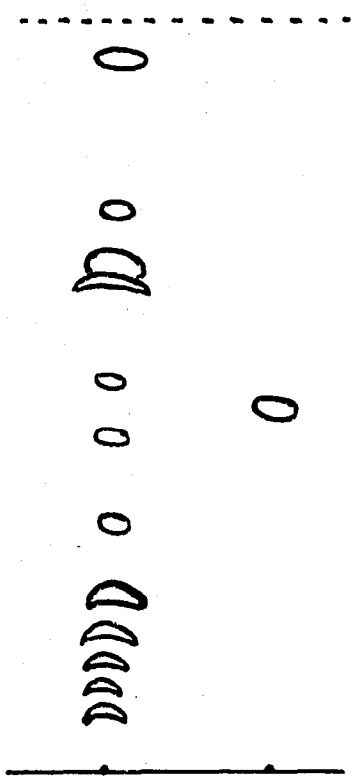

Fig. 4.

Figs. 1–4. Chromatograms of rabbit urine after treatment with chlorpromazine (Fig. 1–I), chlorprotixene (Fig. 2–I and III), imipramine (Fig. 3–I) and amitriptyline (Fig. 4–I). Figs. 1–II, 2–II, 3–II, 4–II show the results obtained with normal rabbit urine to which chlorpromazine (Fig. 1–II), chlorprotixene (Fig. 2–II), imipramine (Fig. 3–II) and amitriptyline (Fig. 4–II) respectively were added.

In the urine of rabbits treated with CPZ, CPX, IP and ATL (100 mg/kg p.o.), 9, 12, 12 and 12 fractions respectively, were detected (Figs. 1–4).

Preliminary experiments indicated the feasibility of performing on the plates some particular chemical reactions designed to establish the nature of the main biotransformation products. This technique can also be applied to other biological fluids or tissue extracts, as well as to other chemically related psychotropic drugs.

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Rapid method for permanent recording of thin-layer chromatograms

One of the most important recent advances in microanalytical technique has been the development of thin-layer chromatography[1]. The inconvenience of handling and storing finished thin-layer chromatograms, however, has made desirable a means of permanently recording the information obtained. Manual tracing on paper, the simplest method in use, yields at best only an approximate reproduction. Shadowgraphing on photographic paper produces an exact negative replica but requires darkroom facilities unavailable in many laboratories. Moreover, photographic processing is time consuming. In this communication a method of duplication is described which eliminates the undesirable features of wet processing through the use of dry process Diazo* paper. By this method positive replicas of thin-layer chromatograms can be made quickly and cheaply under ordinary laboratory conditions with no special equipment or chemicals.

In Fig. 1 are shown a thin-layer chromatogram and its DRIPRINT replica prepared in the following manner. The chromatogram was placed horizontally over a desk lamp equipped with two cylindrical 15 W fluorescent bulbs, the inverted shade serving as a support for the glass plate. A piece of cellophane laid over the coated surface of the chromatogram protected the surface from abrasion by the paper, the paper from chemical attack by residual spray, and functioned as a negative (Fig. 1) for recording such information as the location of the origin and labels identifying the substances applied. A sheet of DRIPRINT paper was then placed over the cellophane followed by a glass plate to hold the various layers stationary. After exposure of the paper to the light for 10 min it was placed in a covered glass jar containing an open

* Diazo paper is a direct positive blueprinting paper. The brand used was DRIPRINT HC 241B (F speed) supplied by Eugene Dietzgen Co., 407 10th St., N. W., Washington, D. C. A sheet 8 in. × 10 in. costs about 1/2 cent. The paper can be handled freely in ordinary laboratory light.