

MULTIPLE SPOT PHENOMENA USING THIN LAYER CHROMATOGRAPHY OF PURE ORGANIC BASES

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Thin layer chromatographic behaviour of some sympathomimetic amines both alone and in the presence of acids in neutral and acidic solvent systems is reported. Thin layers of cellulose, with and without adhesive, alumina and silica gel were used; multiple spot formation only occurred with cellulose thin layers.

It has previously been shown that paper chromatography of amine salts (or of amines in the presence of an acid stronger than those of the solvent system) in neutral or acidic solvents may lead to two spots. One is of the amine in the same position as if chromatographed as the base, the other is present as a portion of the acid spot derived from the anion of the salt (Beckett, Beaven and Robinson, 1960).

The present investigation examines the possibility of multiple spot formation from a pure substance, using thin layer chromatography and several types of thin layers.

EXPERIMENTAL METHODS

Materials. As described in the previous paper (Beckett, Beaven and Robinson, 1960) and also histamine phosphate having m.p. 128.5° [130°, after sintering 127° (Heilbron, Bunbury, 1943)]. Isoprenaline sulphate m.p. 128° [128° (Heilbron, Bunbury, 1943)]. Ephedrine sulphate m.p. 244° [245° decomp. (Heilbron, Bunbury, 1943)]. Ephedrine hydrochloride m.p. 219° [216°–220° (Heilbron, Bunbury, 1943)].

Solvent systems. (i) n-Butanol:acetic acid:water as 4:1:5 by volume. The organic layer was used. (ii) n-Butanol saturated with water.

Spray reagents. As described previously (Beckett, Beaven and Robinson, 1960). Ninhydrin 0.2 per cent w/v in n-butanol was used to detect histamine.

Preparation of plates. All plates used were 20 × 20 cm. They were dried at 100° for 2–3 hr., stored in a desiccator and reheated at 100° for 10 min. before use.

(i) *Cellulose.* (a) *With adhesive.* 20 g. of MN-cellulose powder 300 G (acc. Stahl) and 100 ml. of water for 5 plates. (b) *Without adhesive.* 20 g. of Whatman ashless cellulose powder mixed with 100 ml. of water for 3 plates.

(ii) *Silica gel.* 30 g. Kiesel-gel G-Merck (acc. Stahl) and 60 ml. of water for 5 plates.

(iii) *Alumina.* 30 g. Alumina oxydatum G-Merck (acc. Stahl) and 60 ml. water for 5 plates.

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General Method

All experiments were made at least in triplicate to ensure that any slight possible differences in thickness of the layer were not giving misleading results. All results were within ± 0.03 of the average figures recorded.

0.1 μ molar quantities were applied to the plates, 1.5 cm. from the bottom edge, as solutions of the pure base or salt in the presence of one and ten equivalents of the added acid.

All chromatograms were run for about 1½ hr., at room temperature, during which time the solvent front advanced 10 cm.

All the R_F values reported were calculated from the advanced edges of the spots.

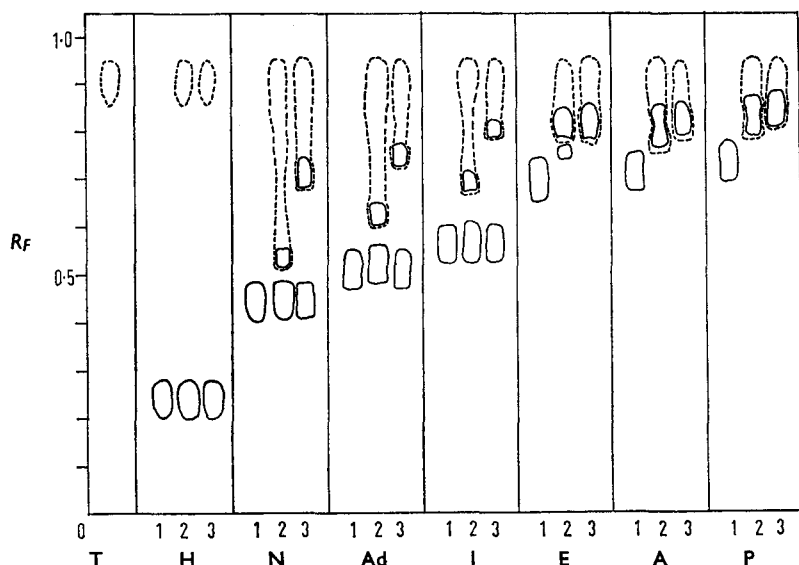


FIG. 1. Thin layer chromatography on cellulose of some sympathomimetic amines developed in the butanol:acetic acid:water solvent system when applied as 1 μ l. of the pure base (or salts) or in solutions with one or ten equivalents of trichloroacetic acid. Continuous outlines indicate the amine, dotted outlines indicate the acid spot. 1. Pure base (0.05 M solution). 2. Pure base plus an equivalent amount of acid (0.05 M solution). 3. Amine (0.05 M solution) plus ten equivalents of acid. T, trichloroacetic acid. H, histamine. N, noradrenaline. Ad, adrenaline. I, isoprenaline. E, ephedrine. A, amphetamine. P, β -phenethylamine.

RESULTS

(i) Cellulose Plates

(a) *With adhesive.* The results using the n-butanol-acetic acid-water system and various amines of different R_F values in the absence and in the presence of 1- and 10-equivalents of trichloroacetic acid are shown in Fig. 1. The observed R_F values under these conditions are summarised in Table I.

TABLE I

R_F VALUES OF SOME SYMPATHOMIMETIC AMINES WHEN APPLIED TO CELLULOSE THIN LAYERS IN THE PRESENCE OF TRICHLOROACETIC ACID USING THE BUTANOL:ACETIC ACID:WATER SYSTEM

Amine	R_F	R_F of two amine spots (1-equivalent of acid present)		R_F of two amine spots 10-fold excess of acid present)	
Histamine	0.27	0.27	—	0.27	—
Noradrenaline	0.47	0.47	0.55	0.47	0.74
Adrenaline	0.55	0.56	0.65	0.55	0.77
Isoprenaline	0.60	0.61	0.71	0.60	0.82
Ephedrine	0.74	0.76	0.84	—	0.85
Amphetamine	0.75	—	0.83	—	0.86
β -Phenethylamine	0.77	—	0.87	—	0.88
Trichloroacetic acid	0.93	—	—	—	—

Similar experiments using dichloroacetic and picric acid as added acids yielded results shown in Fig. 2 and Fig. 3 respectively.

The results using the n-butanol:water saturated system and the above amines in the absence and in the presence of trichloroacetic acid are shown in Fig. 4 and for dichloroacetic and picric acid in Fig. 5 and Fig. 6 respectively.

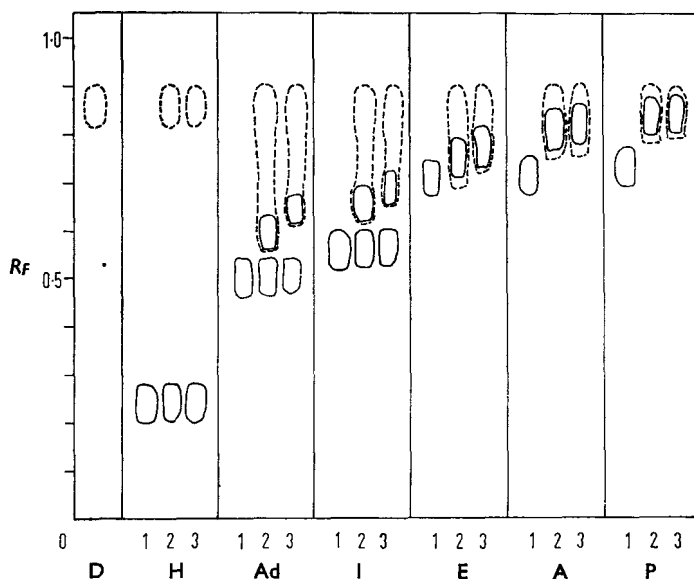


FIG. 2. As FIG. 1 but using dichloroacetic acid (D) instead of trichloroacetic acid.

(b) *Without adhesive.* The experiment was only carried out using the n-butanol:acetic acid:water system and a few amines in the absence and presence of 1- and 10-equivalents of trichloroacetic acid.

Double spot phenomena, similar to the above were obtained, but the R_F values were different, e.g. adrenaline alone had R_F 0.70 but gave two

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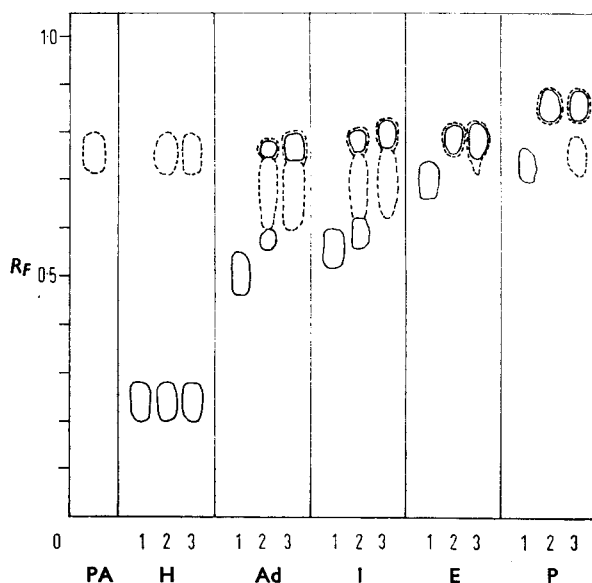


FIG. 3. As FIG. 1 but using picric acid (PA) instead of trichloroacetic acid.

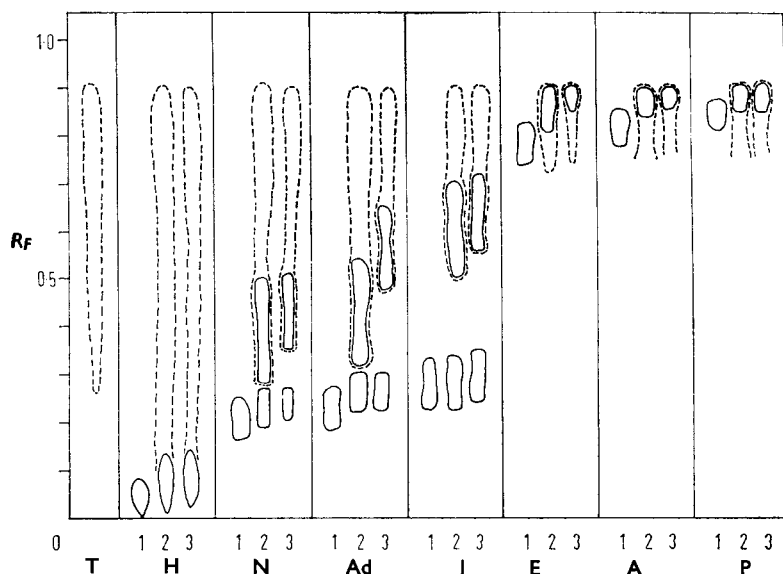


FIG. 4. As FIG. 1 but using water-saturated butanol instead of the butanol:acetic acid:water system.

spots of adrenaline of R_F 0.70 and 0.83 in the presence of either 1- or 10-equivalents of trichloroacetic acid; isoprenaline alone had R_F 0.73 and gave two spots R_F 0.73 and 0.85 with 1- or 10-equivalents of trichloroacetic acid. Trichloroacetic acid alone gave a spot of R_F 0.95.

(ii) *Silica Gel Plates*

The results using the n-butanol:acetic acid:water system are shown in Fig. 7. Although the added trichloroacetic acid caused the amine spot to be elongated, two amine spots were not obtained.

In the butanol:water system the amine only moved slightly from the base line. The addition of 1- or 10-equivalents of trichloroacetic acid did not cause multiple spots of the amines.

(iii) *Alumina Plates*

The results using the n-butanol:acetic acid:water system are shown in Fig. 8; the addition of trichloroacetic acid did not cause multiple spot formation.

In the butanol-water system the amine spot remained in the base line.

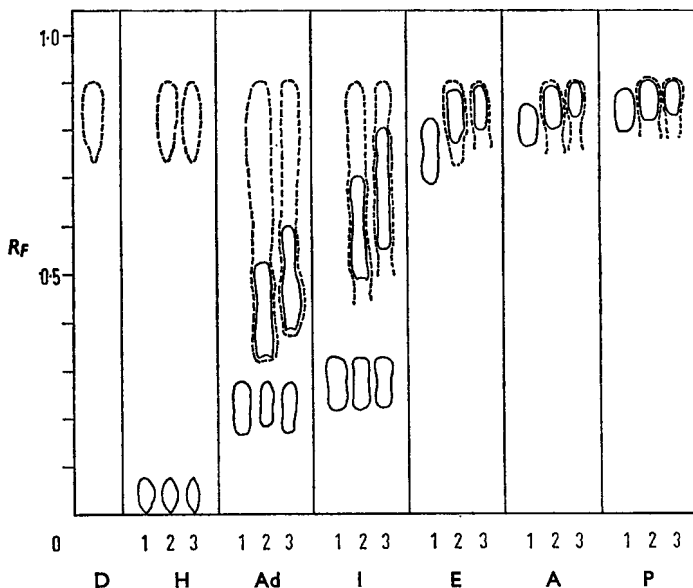


FIG. 5. As FIG 1 but using water-saturated butanol instead of the butanol:acetic acid:water system and dichloroacetic instead of trichloroacetic acid.

DISCUSSION

The results indicate that, just as in paper chromatography, it is possible to obtain two amine spots of an amine salt (or pure amine in the presence of one or more equivalents of acid) when a neutral or weakly acidic solvent system is used in thin layer chromatography involving cellulose as the thin layer.

This pattern of the multiple amine spots under the various conditions is similar to that observed in paper chromatography; detailed discussion is therefore unnecessary since the various factors leading to multiple spot formation in paper chromatography have been already presented (Beckett, Beaven and Robinson, 1960).

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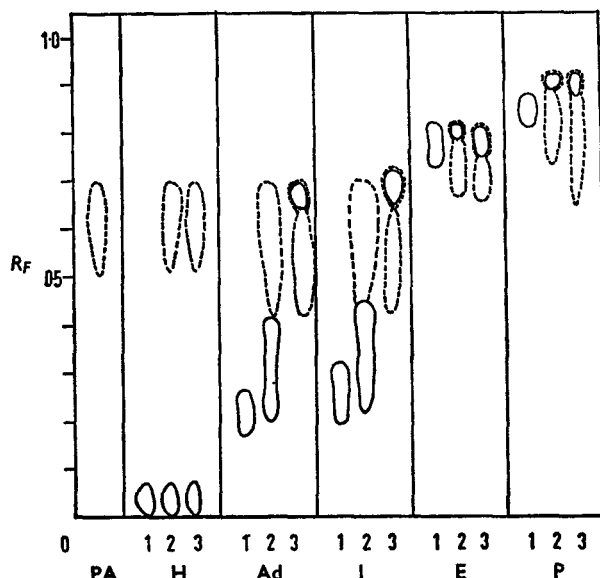


FIG. 6. As FIG. 1 but using water-saturated butanol instead of the butanol:acetic acid:water system and picric acid instead of trichloroacetic acid.

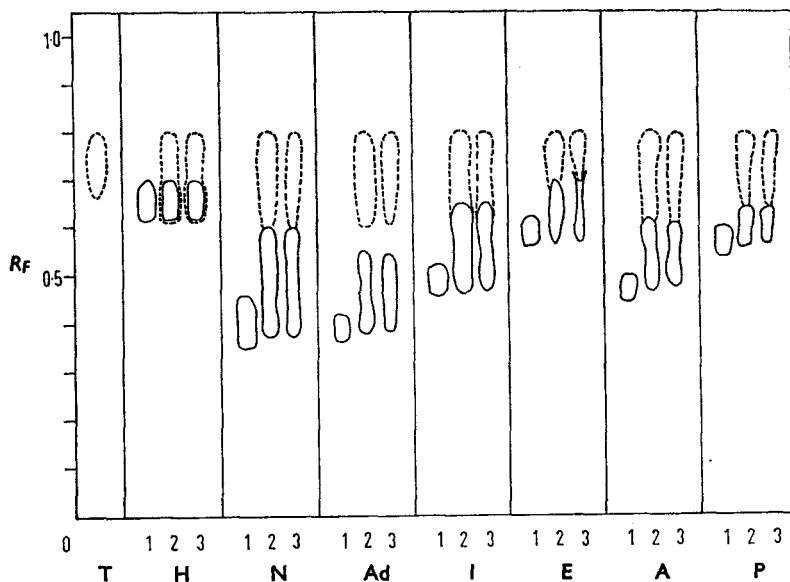


FIG. 7. Thin layer chromatography on silica gel of some sympathomimetic amines developed in butanol:acetic acid:water solvent system when applied as $1 \mu\text{l}$. of the pure base (or salt) or in solutions with one or ten equivalents of trichloroacetic acid. Continuous outline indicates amine spot, dotted outline indicates the acid spot. 1. Pure base (0.05 M solution). 2. Pure base plus an equivalent amount of acid (0.05 M solutions). 3. Amine (0.05 M solution) plus a ten equivalent of acid.

The importance of the role of the added acid and the dependence of the formation of two spots of the amine upon the relative R_F of amine and added acid is depicted in Fig. 9 in which the importance of the equivalent concentration ratio of added acid to base may be seen by comparing Fig. 9 and Fig. 10.

Because a thin layer of cellulose powder without adhesive also gives multiple amine spots from a pure amine, the adhesive is not responsible for the phenomena.

The results, using a thin layer of silica gel and the butanol:acetic acid:water system, are completely different from the above in that only a single spot of an amine is obtained from a pure amine under conditions which would give two spots using cellulose (e.g. Fig. 7, Fig. 1). However the amine spots, using silica gel, are elongated by the presence of one or ten equivalents of added acid (Fig. 7). Since on silica gel, using a neutral solvent system, butanol:water, the amine spots and the acid spots move very little from the base line, no conclusion on the interaction of base with added acid can be made.

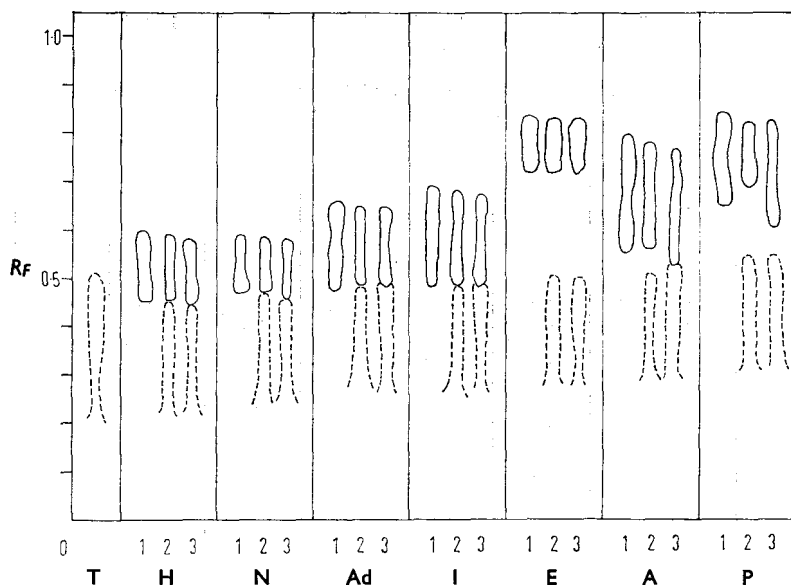


FIG. 8. Thin layer chromatography on alumina of some sympathomimetic amines developed in butanol:acetic acid:water solvent system when applied on 1 μ l. of the pure base or salt or in solutions with one or ten equivalents of trichloroacetic acid. Continuous outline indicates the amine, dotted outline indicates the acid spot. 1. Pure base (0.05 M solution). 2. Pure base plus an equivalent amount of acid (0.05 M solutions). 3. Amine (0.05 M solution) plus ten equivalents of acid.

With a thin layer of alumina and the butanol:acetic acid:water system, the amine spots are unaffected by the presence of one equivalent of added acid and only slightly affected by ten equivalents of acid (Fig. 8). Conclusions cannot be drawn using the butanol:water system, since amines and acids remained on the starting line.

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In thin layer chromatography with alumina, adsorption forces operate exclusively; using silica gel, partition effect will in general be more important than adsorption forces; in a thin layer of cellulose, both partition and adsorption forces exist and their relative role depends upon the conditions.

Since added acid has a negligible effect on the running of amines on alumina, the effect of simple adsorption does not account for the multiple spots of amines on cellulose.

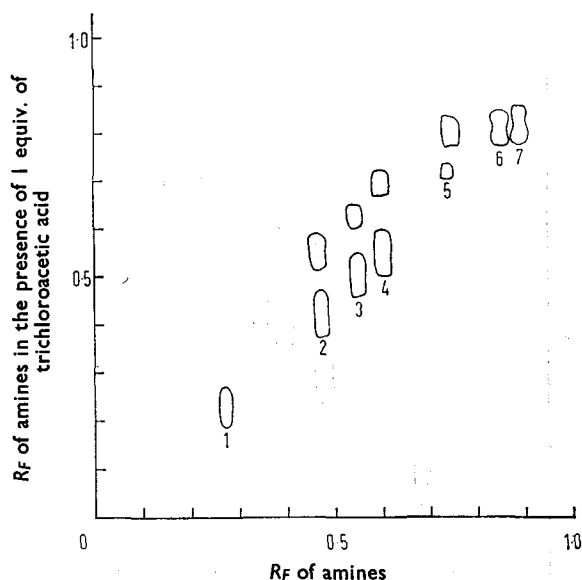


FIG. 9. Thin layer chromatography on cellulose of certain amines run in the butanol:acetic acid:water (4:1:5) solvent system when applied in the presence of 1 equiv. of trichloroacetic acid. 1. Histamine. 2. Noradrenaline. 3. Adrenaline. 4. Isoprenaline. 5. Ephedrine. 6. Amphetamine. 7. β -Phenethylamine.

On silica gel plates, the added acid, even in one equivalent ratio to the amine, has a pronounced effect on the running of the latter. It therefore seems probable that the added acid, as it moves along the plate, has a substantial effect on the partition of the amine between the running solvent and the stationary phase. However, two spots of the amine are not produced.

It is therefore suggested that the formation of two spots of amine, when an amine salt is subjected to thin layer chromatography on cellulose, results from the presence of the carboxy groups in the cellulose. The acid from the anion of the salt, in its movement along the plate, alters the partition of the amines between running solvent and stationary phase and the carboxyl groups make their contribution to the binding of amine (probably anion-cation association) in addition to the non-specific adsorption forces of the cellulose.

It seems that a continuity of adsorption forces along the direction of the running solvent is necessary for the multiple-spot phenomena of amine salts to occur, since two bands of amine do not result when the solutions and solvents are passed down in a column of cellulose under conditions which would give two spots using paper chromatography or thin layer chromatography on cellulose.*

The movement of the amines and acids down a column of non-bound cellulose is from one cellulose particle to another through a layer of solvent. With cellulose adhesively bound to glass in thin layers there is a continuity of adsorption forces.

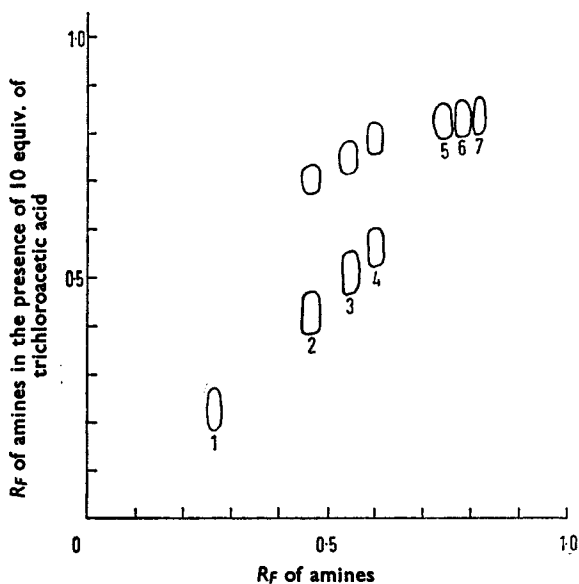


FIG. 10. Thin layer chromatography on cellulose of certain amines run in the butanol:acetic acid:water (4:1:5) solvent system when applied in the presence of 10 equiv. of trichloroacetic acid. Numbers as for Fig. 9.

The present investigation indicates that the presence of two amine spots in thin layer chromatography involving cellulose does not necessarily prove that two amines are present in the solution chromatographed. As in paper chromatography (Beckett, Beaven and Robinsin, 1960), especial care is therefore needed in interpreting information from thin layer cellulose chromatograms of amines in biological fluids or amines in solutions containing acids or salts.

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* Beckett, A. H. and Choulis, N. H. Adsorption forces in cellulose leading to multiple spot formation from pure amines using paper and thin layer chromatography. Paper to be presented at 23rd International Congress of Pharmaceutical Sciences, Munster, September, 1963.

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