THE EFFECT OF TRICHLOROACETIC ACID ON THE PAPER CHROMATOGRAPHY OF TISSUE AMINES

BY B. ROBINSON AND D. M. SHEPHERD

From the Department of Pharmacology and Therapeutics, University of St. Andrews, Queen's College, Dundee

Received February 8, 1961

The nature of the artifacts formed in the paper chromatography of solutions of catechol amines, histamine, and various tryptamine derivatives in trichloroacetic acid (TCA) has been considered. With TCA solutions of histamine the artifact is not the corresponding *N*-acyl derivative. It is suggested the production of these artifacts is assisted by the formation of molecular complexes between the above compounds, acting as electron donors, and the TCA acting as electron acceptor. A solvent is described which eliminates multiple spot formation when TCA solutions of the above type are subjected to paper chromatography.

WHEN solutions of histamine in trichloroacetic acid (TCA) are chromatographed in the solvent system butanol: acetic: water, two spots are obtained, one corresponding to the histamine base and the other to an artifact (West and Riley, 1954). These workers found that the formation of the artifact was not due to a pH effect, but depended on the concentration of the TCA, the concentration of histamine, and on the presence of the basic amino-acids arginine, lysine or ornithine. They suggested that the ability of TCA to extract histamine from tissues might in part be due "to its participation in a loose complex containing histamine (probably as the trichloracetate) and basic amino-acids", and we assume, therefore, that the artifact was thought to be such a complex.

Beckett, Beaven and Robinson (1960) have shown that TCA solutions of many sympathomimetic amines yield two spots on chromatography, the upper spot being closely associated with the TCA spot. It would seem likely that the histamine artifact spot arises in a similar manner, but its exact nature is not clear. Some further observations on the nature of the histamine artifact are now presented.

EXPERIMENTAL METHODS

General Method

Solutions of the amines were applied to Whatman No. 4 paper as indicated in Table I. Ascending chromatograms were run for 18 hr. during which time the solvent front moved about 25 cm. at 18–22°.

Solvents. (1) A single phase mixture of n-butanol: acetic acid: water (12:3:5 v/v). (2) A solution of TCA (1 per cent w/v) in (1), and (3) A solution of TCA (5 per cent w/v) in (1).

After preparation the solvents were allowed to stand 4 hr. before use. Solvent (2) separated into two phases after 3 days, and could if necessary be used several times; solvent (3) separated after 30 hr., and so could be used only once.

Spray reagents. For catechol amines run in solvent (1): a 1 per cent w/v aqueous solution of potassium iodate followed by heating of the

sprayed chromatogram at 100°. For catechol amines run in all solvents: an aqueous buffered solution of potassium ferricyanide (James, 1948) followed by treatment with ammonia, heating at 100° for 3 min. and irradiation with ultra-violet light. The use of this second spray for the detection of catechol amines was particularly necessary after using solvents (2) and (3) as there was appreciable lowering of the sensitivity of the potassium iodate reagent in these instances, presumably owing to the presence of TCA on the paper; the sensitivity of the potassium ferricyanide reagent was unaffected by this latter acid. For tryptamine and

TABLE I

SINGLE SPOT FORMATION BY SOLUTIONS OF AMINES IN 0·01 N HYDROCHLORIC ACID OR 10 PER CENT AQUEOUS TCA SOLUTION AFTER CHROMATOGRAPHY IN A SOLVENT CONTAINING TCA

Solution	Concentration (mg./10 ml.)	Amount of compound applied to paper (μg.)	R_F values in Solvent (3)
Histamine in A	10	100	0·61*
Histamine in B	50	100	0·62
4(5)-Methylimidazole in A	40	40	0·76
4(5)-Methylimidazole in B	40	40	0·76
Imidazole in A	40	120	0·68
Imidazole in B	40	120	0·72
5-Hydroxytryptamine in A	25	75	0·67
5-Hydroxytryptamine in B	25	75	0·67
Tryptamine in A	50	50	0·85
Tryptamine in B	50	50	0·87
Adrenaline in A	40	40	0·65
Adrenaline in B	40	40	0·64
Isoprenaline in A	40	40	0·77
Isoprenaline in B	40	40	0·77
Noradrenaline in A	40	100	0·57
Noradrenaline in B	40	100	0·58

A = 0·01 N hydrochloric acid. B = 10 per cent aqueous TCA.
All R_F values are the average of readings from two chromatograms.
* Some streaking.

5-hydroxytryptamine run in all solvents: a 0·5 per cent w/v solution of *p*-dimethylaminocinnamaldehyde in equal volumes of 5 N hydrochloric acid and absolute ethanol. For imidazole derivatives and 5-hydroxytryptamine run in all solvents: the Pauly diazotised sulphanilic acid reagent.

N-*Trichloroacetylhistamine.* A solution of histamine (150 mg.) in redistilled trichloroacetic anhydride (2 ml.), protected from moisture, was heated on a water bath at 80–90° for 3 hr. (longer heating or heating at higher temperatures led to gradual decomposition). The excess trichloroacetic anhydride was then removed under reduced pressure to leave a yellow oil which on trituration with dry ethanol crystallised as white prisms (206 mg.), m.p. 164–166° (with decomposition from 153°). Recrystallisation from dry ethanol gave white prisms, m.p. 165–167° (with decomposition from 153°) (Found: C, 33·3; H, 3·2; N, 16·0. $C_7H_8Cl_3N_3O$ requires C, 32·9; H, 3·1; N, 16·4 per cent).

4(5)-*Methylimidazole.* This was prepared by the method of Bernhauer (1929).

Other compounds used were obtained commercially.

Results and Discussion

The possibility that the histamine artifact spot might arise from the formation of *N*-trichloroacetylhistamine (N-TCAH) has now been investigated by preparing this compound and studying its chromatographic behaviour. When a solution of N-TCAH in 0·01 N hydrochloric acid was chromatographed in solvent (1), one spot (R_F 0·75) was obtained. After elution with 0·01 N hydrochloric acid and re-running in the same solvent, this again had R_F 0·75. Under identical conditions a solution of histamine in 10 per cent aqueous TCA gave two spots (R_F 0·18 and 0·65) corresponding to the base and the artifact, respectively. The latter spot, after elution and re-running as above, now showed R_F 0·18 corresponding to free histamine. The different R_F values and the behaviour on elution and re-running show that the artifact is not N-TCAH.

That the β-aminoethyl side-chain of histamine is not essential for the artifact formation was demonstrated by the observation that double spots were formed on chromatography in solvent (1) of 10 per cent aqueous TCA solutions of imidazole (R_F 0·44 and 0·69) and 4(5)-methylimidazole (R_F 0·55 and 0·73) from both of which the side-chain is absent; with 0·01 N hydrochloric acid solutions of these two compounds only single spots were obtained R_F 0·45 and 0·53 respectively. When the areas corresponding to the artifacts were eluted with 0·01 N hydrochloric acid, each eluate, re-run in the same solvent, gave a single spot characterisitc of the compound in 0·01 N hydrochloric acid.

Among the conditions under which double spot formation may be expected to occur (Beckett and others, 1960) the possibility of complex formation has been considered. It is of interest that double spots are most clearly defined when TCA solutions of sympathomimetic amines, imidazoles and tryptamine derivatives (West, 1959) are chromatographed. This suggests that artifact production in such cases may be assisted by the formation of molecular complexes between these substances, acting as electron donors and the TCA acting as an electron acceptor by virtue of the marked electronegativity of the three chlorine atoms. These complexes would have structures similar to those formed between aromatic compounds and various electron acceptors as described by McGlynn (1958). Further evidence in support of this hypothesis comes from the following observations: (i) solutions of catechol amines, histamine, or 5-hydroxytryptamine in picric acid (a well-known electron acceptor in complex formation) and in trifluoroacetic acid (TFA) form double spots on chromatography in n-butanol:acetic acid: water (Beckett and others, 1960; Shepherd and West, 1952; West, 1959); (ii) artifact formation with sympathomimetic amines in various acid solutions increases in the order mono- $<$ di $<$ trichloroacetic acid (Beckett and others, 1960).

The above results further emphasise the care which must be taken when interpreting paper chromatograms of tissue extracts prepared in aqueous TCA, this acid being widely used owing to its coagulative effect on protein, which leads to clear extracts and so facilitates subsequent work.

Munier (1952) has suggested that artifact formation may be avoided by the use of the same acid in both the chromatography solvent and in

the solution to be chromatographed. Accordingly we have found that solutions of histamine, imidazole, 4(5)-methylimidazole, tryptamine, 5-hydroxytryptamine, adrenaline, noradrenaline, and isoprenaline when chromatographed in solvent (1) to which TCA has been added, each give a single compact spot (Table I). When the chromatography solvent contained 1 per cent TCA (solvent 2), these substances gave single spots from either 0·01 N hydrochloric acid or 10 per cent TCA solutions, although, when the amount of histamine applied to the paper exceeded (25 μg.), extensive streaking occurred in both cases. When the chromatography solvent contained 5 per cent TCA (solvent 3), histamine in amounts up to at least 100 μg. applied from 10 per cent TCA solution gave a single compact spot, but when applied from 0·01 N hydrochloric acid solution in amounts exceeding 50 μg., histamine still gave some streaking. Using solvent (3) the single spot produced by TCA solutions of all the above compounds had approximately the same R_F value as the corresponding artifact spot produced when they were chromatographed in solvent (1).

The use of solvent (3) provides a method for the chromatography of aqueous TCA extracts of tissues without the risk of misinterpreting the chromatograms owing to multiple spot formation by a single compound.

Acknowledgement. One of us (B.R.) thanks the Wellcome Foundation for the award of a Research Fellowship.

REFERENCES

Beckett, A. H., Beaven, M. A., and Robinson, A. E. (1960). *J. Pharm. Pharmacol.* **12**, *Suppl.*, 203*T*–216*T*.
Bernhauer, K. (1929). *Z. physiol. Chem.*, **183**, 67–73.
James, W. O. (1948). *Nature, Lond.*, **161**, 851–852.
McGlynn, S. P. (1958). *Chem. Rev.*, **58**, 1113–1156.
Munier, R. (1952). *Bull. soc. chim.*, Fr., **19**, 852–873.
Shepherd, D. M., and West, G. B. (1952). *Nature, Lond.*, **169**, 797.
West, G. B. (1959). *J. Pharm. Pharmacol.*, **11**, 595–599.
West, G. B., and Riley, J. F. (1954). *Nature, Lond.*, **174**, 882–883.