A NOTE ON THE USE OF CELLULOSE PHOSPHATE CATION-EXCHANGE PAPER FOR THE SEPARATION OF CATECHOLAMINES, AND SOME OTHER BIOGENIC AMINES

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Cellulose phosphate cation-exchange paper has been investigated for its ability to separate biogenic amines. Descending chromatography with an ammonium acetate:isopropanol solvent mixture gave good separation of a number of these amines, including the principal catecholamines. The technique has the advantage of eliminating neutral and acidic compounds which may interfere in conventional partition methods. It has been applied to tissue extracts containing catecholamines.

THE separation of noradrenaline, adrenaline and other catecholamines by filter paper chromatography was first described by James (1948). Subsequent extensive application of the technique to tissue extracts has, however, revealed some limitations and certain pitfalls for the unwary. As Vogt (1959) explains in a recent symposium, one is liable to get contamination of catecholamines, both with amino-acid precursors such as dihydroxyphenylalanine, and with other biologically active amines such as histamine, in the commonly employed phenol:hydrochloric acid solvent system. Some of the acid metabolites, for example dihydroxyphenylacetic acid, are also not too well separated in this system. There are ways, of course, of circumventing these difficulties by the use of specific chemical reactions, or of specific pharmacological antagonists in a biological type of assay. But it would be an advantage to have available other means of paper chromatographic separation for these amines, if only to increase the certainty of identification of doubtful compounds in tissue extracts.

An obvious approach would be to utilise some of the recently developed cation-exchange papers, which should adsorb basic compounds much more strongly than neutral or acidic precursors and metabolites. Two types are now available: (*a*) cellulose impregnated with resins, and (*b*) modified celluloses such as cellulose phosphate, cellulose citrate and carboxymethyl cellulose. Group (*a*) appears to adsorb catecholamines too powerfully to achieve much individual separation, but cellulose phosphate paper in the second group has many properties that should make it a useful additional tool in this increasingly complex field. It is as easy to use as ordinary filter paper, and separations can be obtained in aqueous salt solutions. However, the rate of irrigation with purely aqueous solvents is rather rapid, and slowing the rate by addition of water-miscible organic solvents gives sharper separations and more compact spots. The pH of the solutions used, and the nature and proportions of the organic solvent mixed with them, influence the R_F value of a compound. The increased flexibility implied by this wide possible

range of combinations of different pH, organic solvent, salt concentration, etc., promises to give the method advantages over ordinary filter paper chromatography. In this preliminary note, results with one solvent system only (ammonium acetate : isopropanol) are reported.

EXPERIMENTAL

Cation-exchange paper: cellulose phosphate paper in the ammonium form (Whatman P20, Reeve Angel and Co. Ltd., London, E.C.4) was cut into 3 × 45 cm. or 3 × 55 cm. strips, and used without pre-treatment for descending irrigation in a standard cylindrical chromatography tank (Aimer Products Ltd., London). The manufacturers point out that P20 is an experimental product, and one should therefore be on the watch for batch differences in adsorption properties. None has so far been found.

Solvent system. 0·2M ammonium acetate (Analar) was adjusted to pH 6·0 with 1·0N acetic acid, and 2 parts mixed with 1 part (v/v) of isopropanol (Analar). A small beaker of isopropanol was placed in the bottom of the chromatography tank.

Compounds. 20–50 μg. of the compounds listed in Table I were spotted by micropipette from solutions containing 2–5 mg./ml. These included some of the amino-acid precursors of the amines studied, and a number of methylated and acidic metabolites of the catecholamines. Histamine was used as the acid phosphate, 5-hydroxytryptamine as the creatinine phosphate, noradrenaline as the acid tartrate, and most of the other bases and amino-acids as hydrochlorides or the free compound.

Development of chromatograms. When the 3 × 45 cm. strips were irrigated for 6–9 hr., the solvent front moved 28–38 cm., and this was convenient for determination of R_F values (Table I). Longer runs with the 3 × 55 cm.

TABLE I

R_F AND R_{ISO} VALUES IN 0·2M AMMONIUM ACETATE + ISOPROPANOL (2 : 1 v/v), WITH CELLULOSE PHOSPHATE PAPER; (R_{ISO} VALUES IN BRACKETS)

Amines—			*Amino-acids—*		
Histamine	0·02	(0·04)	L-Histidine		0·12 (0·23)
(−)-Noradrenaline	0·18	(0·39)	L-Dihydroxyphenylalanine		0·58
5-Hydroxytryptamine	0·24	(0·53)	DL-Tryptophan		0·60
Dopamine	0·26	(0·54)	DL-Tyrosine		0·61
(−)-Adrenaline	0·32	(0·63)	DL-β-Phenylalanine		0·69
Normetanephrine	0·35	(0·76)	*Acids—*		
Tryptamine	0·43	(0·92)	±-Dihydroxymandelic		0·86
Tyramine	0·40–0·46	(0·90–0·93)	Dihydroxyphenylacetic		0·91
Metanephrine	0·45	(1·00)	±-3-Methoxy-4-hydroxy-mandelic		0·96
(±)-Isopropylnor-adrenaline	0·46	(1·00)	Homovanillic		0·96

strips (16–24 hr.), allowing the solvent to run off the paper, were done in the presence of added isopropylnoradrenaline (20 μg. of isoprenaline sulphate B.P.). This compound was chosen as a "marker," because it travelled the farthest of the catecholamines tried and is probably not a naturally-occurring substance. An "R_{ISO}" value of the compound under test was measured (ratio of the distance travelled by the compound to the

distance travelled by the isopropylnoradrenaline: Table I, figures in brackets).

Detection of compounds on paper. (1) acetone-dip techniques, using ninhydrin and Ehrlich's Reagent (Smith, 1960, pp. 95, 96), and a diazotised *p*-nitraniline spray (Smith, 1960, p. 297: "Nitraniline Reagent II") were applied to the appropriate compounds. (2) A convenient and simple acetone-dip technique, exploiting the fluorescent ethylenediamine reaction, was devised for the detection of the catechols. The cellulose phosphate

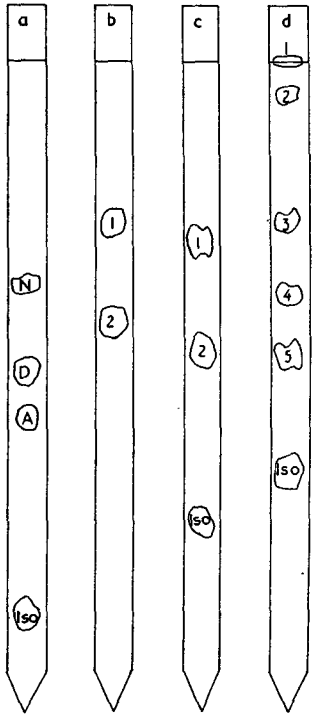

FIG. 1. Cellulose phosphate strips, 3 × 55 cm., run for 18–20 hr. in ammonium acetate-isopropanol; spots located with ethylene diamine and ammonia (for details, see text).

a, Separation of noradrenaline (N, R_{ISO} 0·40), dopamine (D, R_{ISO} 0·56) and adrenaline (A, R_{ISO} 0·64).

b, Ox adrenal extract, showing 2 spots (1 and 2).

c, Ox adrenal extract run with isopropylnoradrenaline; R_{ISO} of spot 1 is 0·38 (consistent with noradrenaline), and of spot 2 0·63 (consistent with adrenaline).

d, Banana peel extract run with isoprenaline. Spots 1, 2 and 5 have R_{ISO} 0·0, 0·08 and 0·72, not consistent with any compounds tried. Spots 3 and 4 have R_{ISO} 0·40 and 0·56, consistent with noradrenaline and dopamine respectively.

strips, allowed to dry at room temperature (15–20°), were dipped in a mixture of 1 part by volume of ethylenediamine (M. & B. Laboratory Chemicals) and 9 parts by volume of Analar acetone. After evaporation of the acetone, the strips were hung overnight in a large glass tank containing a beaker of 0·880 ammonia solution. The papers were then examined under ultra-violet light (Hanovia Chromatolite). Quantities of adrenaline and noradrenaline as low as 0·2 μg. can be detected by this method, even after chromatography. 5-Hydroxytryptamine also gave a strong green fluorescence with ethylenediamine, but the 3-methoxy derivatives reacted only very slowly (48–96 hr.).

RESULTS

R_F and R_{ISO} values. R_F values recorded in Table I show clearly that the compounds separated into 3 main groups: (1) the acids, running

well towards the solvent front (R_F 0·86–0·96); (2) the neutral amino-acids (R_F 0·58–0·69), and (3) the bases. The imidazole derivatives histamine and histidine were strongly adsorbed near the origin. The other, more weakly basic, compounds ran between R_F 0·18 and 0·46. 5-Hydroxytryptamine and dopamine were not well separated in this solvent system. Tyramine, alone among the compounds tried, gave somewhat variable R_F and R_{ISO} values, and therefore a range is quoted in the Table. The 16–24 hr. irrigation, using isopropylnoradrenaline as marker, increased the separation of these bases, and eliminated the neutral amino-acids and acidic metabolites which ran off the paper. An example of the separation of noradrenaline, dopamine and adrenaline is shown in Fig. 1a.

Application to tissue extracts. Some tissues, known to contain catecholamines, were homogenised with 0·4N perchloric acid, and the extracts, after removal of perchlorate as the potassium salt, run on cellulose phosphate paper. Ox adrenal medulla (containing principally adrenaline and noradrenaline) and banana peel (noradrenaline and dopamine, with some 5-hydroxytryptamine) were used as a test of the technique, and typical chromatograms are illustrated in Fig. 1, b, c and d. 5-Hydroxytryptamine was not detected by Ehrlich's reagent in the banana peel extract; it is known to occur in much smaller quantities than noradrenaline and dopamine (Waalkes, Sjoerdsma, Creveling, Weissbach, and Udenfriend, 1958).

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