This method of chromatographic analysis of basic amino acids has been used in this laboratory for several months and has allowed substantial savings in both time and reagents.

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Chromatographic separation of histamine and some metabolites on cellulose phosphate paper*

The metabolism of histamine (M) in animals has been reviewed recently[1]. The main products of metabolism are 1-methyl-4-(β-aminoethyl)-imidazole (MeM), imidazole-4(5)-acetic acid (ImAA), imidazoleacetic acid riboside (ImAA-riboside), 1-methyl-imidazole-4-acetic acid (MeImAA), and acetyl-histamine (AcM). Quantitative analyses of ^{14}C-labelled histamine metabolites have been performed by crystallization of the different products as their respective picrates or *p*-iodophenyl-sulfonyl (pipsyl) salts[2,3]. These methods, however, are quite cumbersome and time-consuming.

Paper chromatography has been used by SCHAYER AND KARJALA[2] to separate radioactive metabolites of histamine. Three radioactive peaks were found using a solvent system composed of 1-butanol–ethanol–ammonia (80:10:30, v/v). The first peak contained ImAA-riboside, the second MeImAA and ImAA, and the third M, MeM and AcM. Good separation of histamine, histidine and acetyl-histamine can be obtained using Whatman No. 1 filter paper with isopropanol–0.25 *N* NH_4OH (3:1)[4]. In this system MeM can not be separated from M nor ImAA from MeImAA[5]. Thus, until now, it has not been possible to separate all of the above mentioned metabolites of histamine by paper chromatography.

In a preliminary effort to separate the different known metabolites of histamine by chromatography, a variety of papers were tried, Schleicher and Schüll papers No. 5984D, 2040A, blue R589D, white R589, 2043A, 2041, red R589, 598, 2043-D, 602 extra dense, 576, green R589, black R589D, 470 and 470A. Also, different solvents (as recommended by BLOCK *et al.*[6]) and ionophoresis at different pH values were evaluated. None of these techniques proved successful. Amberlite ion exchange resin loaded papers (Sa-2, Wa-2, Sb-2 and Wb-2) and Whatman cellulose ion exchangers (P-20, CM-50, DE-20 and ET-20) were tried next. Best results were achieved with the cation exchanger, cellulose phosphate paper (P-20). These results are reported below.

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Experimental procedure

Ascending and descending chromatography with P-20 cellulose phosphate paper was used. To recycle the paper through its H^+ and Na^+ forms we washed it in a downward direction successively with (1) 0.1 *N* HCl, (2) water, (3) 0.1 *N* NaCl, (4) water; each step proceeded for 24 h. To prepare a bi-functional cation exchanger the paper was washed with a solvent containing 0.1 *N* KCl and 0.001 *N* KOH, for 24 h and then washed with water. The washed papers were airdried. The chromatograms were developed with 0.1 *M* phosphate buffer (pH 7.5) for 1.5 h by the ascending technique and 3 h by the descending technique. The solvent front for the former reached 21 cm and for the latter 46 cm. The papers were dried in air and dipped in *p*-nitrobenzene diazonium fluoborate (Eastman No. P7078, 1 g in 100 ml acetone), and then sprayed with 0.1 *N* KOH in ethanol[7]. The stabilized diazotizing reagent was used in place of the recommended diazotized sulfanilic acid followed by Na_2CO_3 (BLOCK *et al.*[6]) because the latter reagent did not react with either MeImAA or MeM.

Results and discussion

Using unwashed cellulose phosphate paper with phosphate buffer, pH 7.5 as solvent, two zones resulted on the paper, one basic, the other acid as visualized with a bromocresol green indicator spray. The basic zone extended from the origin to about R_F 0.3; the acid zone from 0.3 to the solvent front. These zones could also be observed after treatment with the diazotizing color reagent; the lower zone (R_F 0–0.3) was dark brown due to formation of nitrobenzene, while the upper zone was light yellow. The zone formation may be due to non-equilibrium conditions of the ion exchange process or due to the separation of the solvent into two phases during development, as has been observed by POLLARD *et al.*[8].

Formation of two phases, however, did not seem to affect the separation of the compounds. As a matter-of-fact, histidine always migrated to the line of phase separation, giving a slightly distorted spot (see D, Fig. 1). Prewashing of the paper with either the developing buffer, HCl followed by NaOH, or KCl and KOH did not eliminate the two phases on the paper. Using paper washed with KCl and KOH improved the chromatography and facilitated better visualization of the MeImAA spot.

TABLE I

R_F VALUES OF SOME IMIDAZOLE DERIVATIVES* ON CELLULOSE PHOSPHATE

Imidazole derivatives	*Abbreviation*	R_F		*Color of spots (NBDFB reagent)***
		Ascending	*Descending*	
Histidine	D	0.29	0.32	Dark brown
Histamine	M	0.07	0.08	Dark brown
Methyl-histamine***	MeM	0.15	0.15	Yellow
Acetyl-histamine	AcM	0.46	0.48	Orange
Imidazoleacetic acid	ImAA	0.54	0.54	Reddish brown
Methyl-imidazoleacetic acid***	MeImAA	0.68	0.79	Orange

* Experimental details in text.
** 1% (w/v) *p*-nitrobenzene diazonium fluoborate in acetone, followed by 0.1 *N* ethanolic KOH.
*** These compounds were obtained from Dr. R. W. SCHAYER, later purchased from Calbiochem, Los Angeles.

The color of the imidazole derivatives and their R_F values are given in Table I. A chromatogram of the imidazole derivatives is shown in Fig. 1, illustrating excellent separation of 6 compounds. To obtain even better separation of AcM and ImAA a chromatogram had to be run for 6 h, and the solvent was allowed to drip off the bottom of the paper.

ImAA-riboside could not be obtained for these studies, but its identity on paper probably could be determined by chromatography after acid hydrolysis (1 *N* HCl at 150°, for 6 h) as ImAA[9].

Although excellent separation of histamine derivatives is achieved, the method

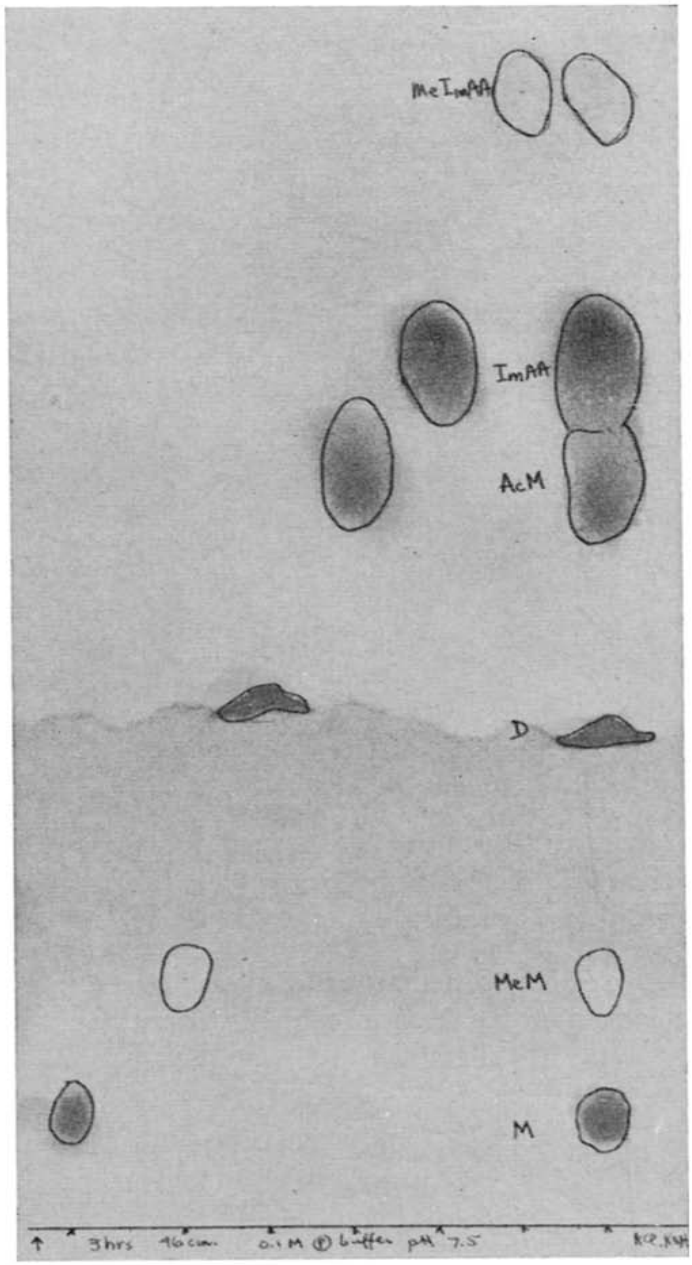

Fig. 1. Paper chromatogram of imidazole derivatives. P-20 cellulose phosphate paper; development time 3 h with 0.1 *M* phosphate buffer, pH 7.5, as solvent; descending development. D = histidine, all other abbreviations in text.

is not suitable for micro-amounts since the least detectable amount is *ca.* 50 μg for all investigated derivatives except for MeImAA for which *ca.* 100 μg is necessary. In contrast, only 1.0–10.0 μg quantities produce colored spots on regular cellulose paper.

Using the above technique, quantitative analysis of ^{14}C-histamine metabolites from embryonating chicken eggs is being investigated. Metabolic radioactive products are chromatographed by the described procedure with known compounds as markers, and the developed spots cut out and their radioactivity measured with a liquid scintillating spectrometer[10].

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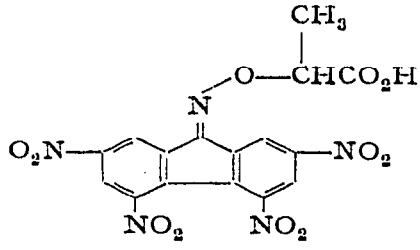
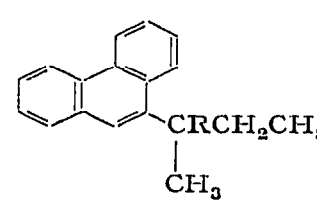
Optical resolution of 9-sec.-butylphenanthrene by molecular complexation chromatography

KLEMM AND REED[1] described the use of columns of silicic acid impregnated with the optically active molecular complexing agent (+)- or (—)-α-(2,4,5,7-tetranitro-9-fluorenylideneaminoöxy)-propionic acid (I) for optical resolution of an ether and of an ester containing one center of asymmetry (on carbon) for each compound. In addition the method was used for optical resolution of a hydrocarbon wherein optical activity



(I)



(II): R=H
(III): R=OH