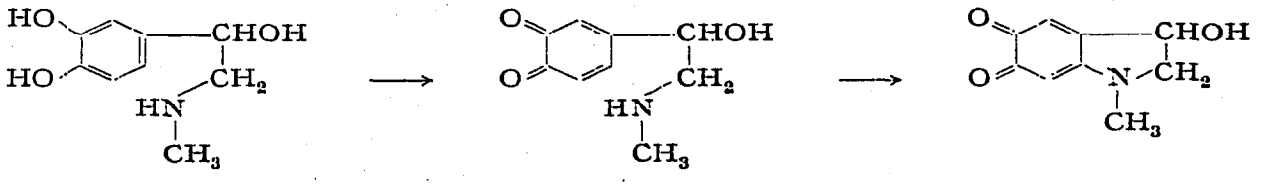
Short Communications

Detection of biogenic amines on paper chromatograms

The detection of adrenaline and noradrenaline on paper chromatograms can be carried out by the method proposed by JAMES[1,2], in which the chromatograms are sprayed with a 0.44% potassium ferricyanide solution in phosphate buffer of pH 7.8.

A red brown or light red spot is formed, due to the formation of an indole derivative, adrenochrome:



This reaction is given by other arylaliphatic amines that possess a secondary amino group in β position in the side chain, have no substituents in the *ortho* position and contain a catechol group.

By means of this reaction not only adrenaline but also several related amines can be detected on paper chromatograms as already reported by CRAWFORD[3].

GOLDENBERG *et al.*[4] observed that this reaction could be enhanced if the chromatograms were subsequently sprayed with a solution of ferric chloride. In this case the brown spots turn blue, the sensitivity becoming much higher and visualization easier.

This reaction does not depend on the formation of adrenochrome, but only on the ability of the various substances to reduce the potassium ferricyanide to ferrocyanide at room temperature and on paper. The ferrocyanide can then be easily detected with ferric salts, with which it forms an insoluble and deeply coloured precipitate (prussian blue).

We have observed that, owing to its great sensitivity, this second reaction can be used to detect a series of amines and amino acids of biological and pharmaceutical interest that cannot be revealed by the adrenochrome reaction, *viz.* 1-(3-hydroxyphenyl)-2-ethylamino-ethanol (Effortil), 1-(4-hydroxyphenyl)-2-methylamino-ethanol (Sympathol), tyramine, tyrosine, tryptamine, 5-hydroxytryptamine (Serotonin), tryptophan, 5-hydroxytryptophan.

Furthermore this reaction enables us to distinguish rapidly the amines that on oxidation yield products of the adrenochrome type (adrenaline, noradrenaline, isoprenaline (1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanol), 3-hydroxytyramine, 3-hydroxytyrosine (DOPA), etc.) from those that do not give such products.

We have found that the use of an unbuffered solution of potassium ferricyanide before the treatment with the ferric salt solution makes it possible to detect many amines that are not revealed with the ferric salt if the buffered spray reagent is used (Tables I and II).

TABLE I

REACTION OF AMINES WITH UNBUFFERED FERROCYANIDE FOLLOWED BY FERRIC CHLORIDE

Solvent: butanol–acetic acid–water (4:1:5); development time: 12 hours.

Substance	R_F	*Red colour with* $K_3Fe(CN)_6$ *0.5% in* H_2O	*Sensitivity* μg	*Blue colour with* $FeCl_3$ *0.5%*	*Sensitivity* μg
Adrenaline	0.36	+	2	+	0.2
Noradrenaline	0.29	+	5	+	0.1
Sympathol*	0.53	—		+	0.2
Effortil	0.72	—		+	0.2
Isoprenaline	0.58	+	0.3	+	0.1
Tyramine	0.63	—		+	0.5
3-Hydroxytyramine	0.39	+	1	+	0.1
Tyrosine*	0.35	—		+	0.3
3-Hydroxytyrosine	0.22	+	0.5	+	0.2
Tryptamine	0.73	—		+	0.1
5-Hydroxytryptamine	0.43	—		+	0.1
Tryptophan	0.41	—		+	0.3
5-Hydroxytryptophan	0.19	—		+	0.1

* After being dipped in $K_3Fe(CN)_6$, the chromatograms were dried in an oven or at room temperature.

TABLE II

REACTION OF AMINES WITH BUFFERED (pH 7.8) FERROCYANIDE FOLLOWED BY FERRIC CHLORIDE

Solvent: butanol–acetic acid–water (4:1:5); development time: 12 hours.

Substance	R_F	*Red colour with* $K_3Fe(CN)_6$ *0.44% in phosphate buffer pH 7.8*	*Sensitivity* μg	*Blue colour with* $FeCl_3$ *0.5%*	*Sensitivity* μg
Adrenaline	0.36	+	2	+	0.3
Noradrenaline	0.29	+	5	+	0.1
Sympathol	0.53	—		—	
Effortil	0.72	—		—	
Isoprenaline	0.58	+	0.3	+	0.1
Tyramine	0.63	—		—	
3-Hydroxytryptamine	0.39	+	1	+	0.3
Tyrosine	0.35	—		—	
3-Hydroxytyrosine	0.22	+	0.5	+	0.1
Tryptamine	0.73	—		—	
5-Hydroxytryptamine	0.43	—		+	0.2
Tryptophan	0.41	—		—	
5-Hydroxytryptophan	0.19	—		+	0.3

The sensitivity of the reaction was established by chromatographing solutions of each substance in the following concentration range: 5, 2, 1, 0.5, 0.3, 0.2, 0.1 μg per spot.

To carry out the reaction the chromatogram was dipped for a few seconds in a solution of potassium ferricyanide and after 10 minutes the sheet was rapidly

dipped in a solution of ferric chloride (0.5 %). Blue spots formed on a yellow background; the chromatograms are not stable due to the diffusion of the blue colour, which is less rapid if the sheets are dried before being dipped in ferric chloride solution.

The reaction is positive also with other substances, such as pyrocatechol, resorcinol, hydroquinone[5] and ascorbic acid, and may be used for detecting other reducing compounds.

Further applications of this reaction will be reported in due course.

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A sample-solvent evaporator for paper chromatography

In preparing a chromatogram before development in a suitable solvent system, one applies a liquid sample along a starting line, or as a spot, with a pipette held in one hand and with the tube containing the sample, as well as a source of air, usually a glass pipette connected to a compressed air line, for evaporating solvent as the sample is applied to the paper, held in the other hand. This procedure is unwieldy and offers several possible sources of difficulty. For one thing, there may occur excessive spread of the starting line band resulting in wide bands, or excessively spread spots, on the finished chromatograms making analysis difficult if not impossible. When one attempts to restrict the spread of the starting line band by applying the air jet to the paper immediately after applying the sample, there is danger that the air jet will cause the spray of sample from the tip of the application pipette to the paper. This results in annoying extraneous spotting of the chromatogram. At best, it is difficult to apply such liquid samples in a uniform manner and without excessive spread of the starting line band.

This report describes an apparatus which circumvents the difficulties described by allowing efficient continuous evaporation of the solvent contained in a sample which is applied to the starting line of various widths of chromatograms. This unit is presented in Fig. 1. It is made of a section of ¼ in. (O.D.) brass tubing plugged at one end and into which are drilled holes of 0.040 in. diameter, spaced ¼ in. apart. Sliding sleeves of ¼ in. (I.D.) permit any number of holes to be exposed depending upon the width of chromatogram to be processed. The open end of the tubing is connected to a compressed air line, or to a nitrogen line, and air is thus allowed to