pipecolinic acid, baikiain (Δ^4-tetrahydropyridine-2-carboxylic acid) and sarcosine show up as red spots after about six hours standing at room temperature and reach maximum intensity after standing overnight. β- and γ-pipecolinic acids give comparatively faint colours.

The complete range of commercially obtainable α-amino-acids gave either no colour or indefinite faint browns, with the exception of glycine, which gave a quite strong greenish-brown. This colour, however, is not easily distinguishable from the faint browns given by many amino-acids, and hence vanillin is not recommended as a glycine reagent. β-Alanine, β-amino-*iso*-butyric acid, γ-amino-*iso*-butyric acid, glutathione, betaine, hippuric acid, creatinine, glycocyamine, ethanolamine, pyrrole, pyrrolidine, pyrrolidone and indole also gave negative results. In general, the red spots only fade slightly in a week, though they tend to be obscured by darkening of the background in two or three days. Baikiain is an exception, becoming brown in about 20 hr. The background darkening is rapid after running the chromatograms in phenol, the spots also becoming discoloured. Results are satisfactory after running in butanol–acetic acid, collidine–lutidine or the acetone–urea–water solvent of Whitehead and Bentley[6]. The accompanying table shows the minimum detectable quantities of the substances giving well-defined colours in daylight when run on 15-cm. one-way acetone–urea–water chromatograms.

Sensitivities of Amino-acids to Vanillin

	μgm.		μgm.
Ornithine (30 sec.)	0·5	α-Pipecolinic acid (16 hr.)	1
Sarcosine (16 hr.)	0·5	Baikiain (16 hr.)	1
Proline (16 hr.)	1	β-Pipecolinic acid (16 hr.)	4
Hydroxyproline (16 hr.)	1	γ-Pipecolinic acid (16 hr.)	5

The use of a range of aldehydes and ketones as specific amino-acid chromatographic colour reagents is being investigated and will be described later.

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Detection of 5-Hydroxytryptamine by Paper Chromatography

It is well known that enterochromaffin cells acquire a golden-yellow fluorescence after fixation in formaldehyde, due probably to their content of enteramine (5-hydroxytryptamine). We have now carried out chromatographic experiments with synthetic 5-hydroxytryptamine creatinine phosphate to study the sensitivity and specificity of this reaction. We used a butanol – acetic acid – water solvent and a developer composed of nine parts of a solution of potassium dichromate (0·1 per cent) and one part of formaldehyde solution (37–41 per cent). After spraying and heating at 100–110° C. for five minutes, the paper is viewed under ultra-violet light. Under these conditions, 5-hydroxytryptamine (R_F value 0·38) produces a golden-yellow fluorescence immediately after the heating and this persists for days; quantities as small as 0·2 μgm. base can be detected. The behaviour of bufotenine and bufotenidine is quite similar to that of 5-hydroxytryptamine. Tryptamine gives a bright yellow fluorescence if more than 2·5 μgm. are present; but this develops slowly. Tryptophane likewise gives a yellowish fluorescence if 2·5 μgm. are present, while 5-methoxytryptamine gives a yellow-orange fluorescence if similar amounts are present. Bufothionine, histamine, histidine, tyramine, tyrosine and *p*-norsynephrine produce no fluorescence, while that with adrenaline and noradrenaline is very feeble. *m*-Norsynephrine has already been shown to produce a violet fluorescence under these conditions if 0·5 μgm. is present[1]. Such a small amount of 5-hydroxytryptamine (0·2 μgm.) can also be detected with alkaline Folin reagent (producing a blue colour), Pauly reagent (red), diazotized *p*-nitraniline reagent (red-brown), N.N.C.D. reagent of Heinrich and Schuler (red-brown), Gibbs reagent (brownish-violet) and Ehrlich's reagent (blue)[2].

The fluorescent reaction may therefore be of value in tracing small quantities of 5-hydroxytryptamine in tissue extracts. The diazotized *p*-nitraniline reagent may also assist, since hydroxyindolealkylamines (for example, 5-hydroxytryptamine, bufotenine and bufotenidine) give an orange-brown colour whereas the indolealkylamines (for example, tryptamine) usually give a faint yellowish colour in higher concentrations.

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Quantitative Paper Chromatography of Traces of Metal with the Aid of Radioactive Hydrogen Sulphide

The methods so far known for the determination of traces of metal are often expensive and take much time. Moreover, either the sensitivity is low or the error is large. On the other hand, quantitative paper chromatography has not developed far enough yet to give a competitive solution. I have investigated the possibility of using radioactive hydrogen sulphide ($H_2{}^{35}S$).

The quantitative reaction with radioactive hydrogen sulphide and the oxidation of the sulphides formed a first difficulty. This was solved in the following way. After chromatography, the dried papers are