Peptides and proteins can be detected in minute amounts, although some hydrolysis may occur in the spots, which perhaps limits the method for preparative purposes.

The method should be of a great value also for the detection of other buffering substances which cannot be identified by more specific reactions. A possible generalization of our method would be to treat the paper with a substance the reaction of which is inhibited by the presence of spots of the compounds under examination.

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[1] Woiwod, A. J., *Nature*, **166**, 272 (1950).
[2] Gal, E. M., *Science*, **111**, 677 (1950).
[3] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **176**, 367 (1948).

Separation of Alkaloids by Paper Chromatography

References to the separation of alkaloids on columns by partition chromatography have been made by Catch *et al.*[1] and Partridge[2]. The theory and use of buffered columns have been previously described[3–5]. In the separation of the penicillins, buffered filter paper has been used[6], and the difficulty of obtaining absolute R_F values for such separations has been noted[7]. The water-insoluble alkaloids of ergot have been separated on unbuffered paper[8].

We have successfully used buffered filter paper for the separation of the strongly basic solanaceous alkaloids, and the feebly basic water-insoluble alkaloids of ergot.

In the case of solanaceous alkaloids, separation of atropine and hyoscine occur, and buffers of *p*H 5–9 were used. A weak solution of iodine in potassium iodide and water was used to detect the alkaloid on the paper. *M*/15 buffer was found to be the most suitable strength, and of the buffers tried phosphate and citrate-phosphate gave the best results, while of the various solvents used *n*-butanol gave the nicest separations. The graphical representation of separations obtained at various *p*H values of a mixture of apoatropine, methyl nitroatropine, atropine and hyoscine is shown in Fig. 1. Similar separations can be made of brucine and strychnine; morphine, narcotine, cocaine and the quinine alkaloids give spots of varying R_F, using butanol and buffered paper.

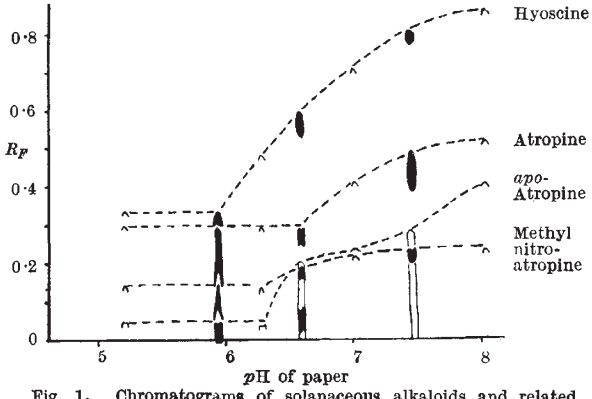

Fig. 1. Chromatograms of solanaceous alkaloids and related products

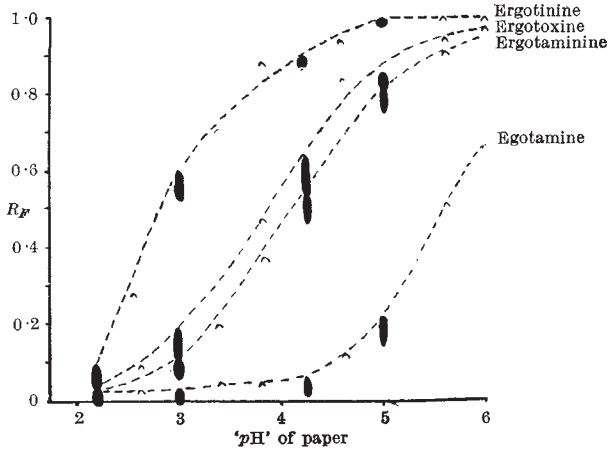

Fig. 2. Chromatograms of water-insoluble alkaloids of ergot

A similar method has been applied to the water-insoluble alkaloids of ergot, with the view of separating the physiologically inactive from the active alkaloids. By the use of buffered filter papers over the range '*p*H 2·2–6', varying degrees of separation of the water-insoluble alkaloids have been carried out. The most satisfactory solvent was diethyl ether saturated with water. In the accompanying graph (Fig. 2) the effect of the '*p*H' of the paper on the R_F value is shown, and the scope of the separations is obvious. With ether as solvent both lysergic and *iso*lysergic acid remain stationary. Ultra-violet light was used to detect the alkaloidal spots.

Quantitative estimations by elution of the alkaloid have not been satisfactory, recoveries varying from 75 to 88 per cent using 15–40 μgm. ergotoxine. These losses are being investigated. No resolution of ergotoxine into ergocristine, ergocornine or ergokryptine was observed in the systems used. Filter-paper chromatography is well suited to the qualitative analysis of the ergot alkaloids in view of the micro amounts used, but for quantitative work more promise is being shown by the use of buffered columns.

It is apparent that by the use of variations in *p*H and different solvents, very complex mixtures of alkaloids should be separable (provided that partition coefficients and/or the *p*K's differ). Thus, valuable information may be obtained for the 'scaling up' of comparable systems for macro separations, the very small amounts of material used in such preliminary investigation being a very desirable feature.

This work will be published in detail and an investigation is proceeding of the quantitative aspects of the separations described.

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[1] Catch, J. R., Cook, A. H., and Heilbron, I. M., *Nature*, **150**, 633 (1942).
[2] Evans W. C., and Partridge, M. W., *Quart. J. Pharm. Pharmacol.*, **21**, 126 (1948).
[3] Synge, R. L. M., *Analyst*, **71**, 256 (1946).
[4] Brit. Pat. 569844.
[5] Levi, A. A., *Biochem. J.*, **43**, 257 (1948).
[6] Goodall, R. R., and Levi, A. A., *Nature*, **158**, 675 (1946).
[7] Baker, D. F., Dobson, F., and Martin, A. J. P., *Analyst*, **75**, 651 (1950).
[8] Foster, G. E., MacDonald, J., and Jones, T. S. G., *J. Pharm. Pharmacol.*, **1**, 802 (1949).