THE SEPARATION AND IDENTIFICATION OF ERGOT ALKALOIDS BY PAPER PARTITION CHROMATOGRAPHY

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During the long history of ergot much time has been devoted to the study of the alkaloids, to which the drug owes its therapeutic and toxic properties. Many workers have contributed to our knowledge of this subject and, in particular, the problem of assaying ergot has been a major topic, engaging the attention of laboratories all over the world. A good summary of analytical work up to 1937 has been given by Barger[1] and a later account has more recently been published by the American Pharmaceutical Association[2]. In spite of the effort already expended on this project, however, available methods still lack specificity; biological assays measure the total potency due to the alkaloids in the preparation under examination, while colorimetric and other chemical methods estimate the total alkaloids, the water-insoluble or the water-soluble alkaloids. The results may be stated in terms of alkaloid calculated as ergotoxine, ergotamine or ergometrine but no method so far available will allow the actual amounts of these alkaloids in a specimen of ergot to be determined.

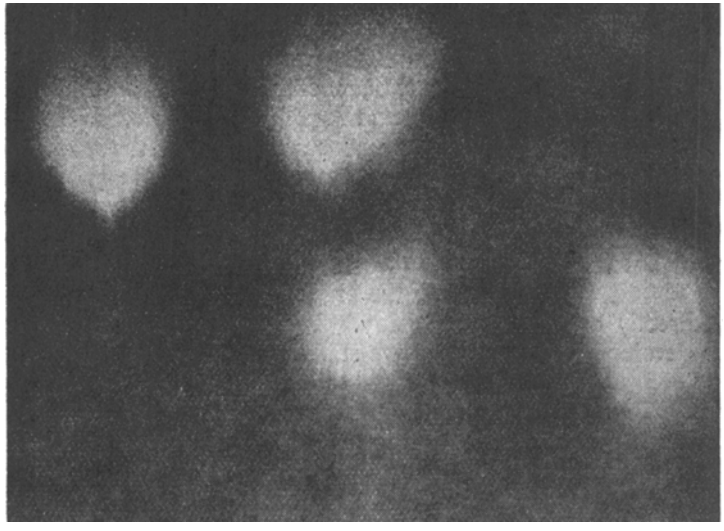
It was the purpose of the work, described in the present communication, to apply the technique of paper partition chromatography[3] to this problem for, in view of the remarkable success of this new technique in other fields, there was good reason to believe that results of interest would emerge.

Separation of Alkaloids

The separation of amino-acids on paper chromatograms using a water saturated solvent, first described by Consden, Gordon and Martin[3], is well known. These workers used strips of filter paper, on which were placed spots of the solutions under test, the upper end of the paper being immersed in a horizontal trough containing the water-saturated solvent. The strips were hung in an airtight chamber in an atmosphere saturated with water and solvent. The solvent from the trough gradually passed down the paper causing separation of the amino-acids which were subsequently located on the paper by use of the ninhydrin reagent. A suitable chamber was provided by using a stoneware drain-pipe standing vertically and closed by a lead tray at the bottom and a sheet of plate glass at the top. Water saturated with solvent was placed at the bottom of the chamber in order to maintain the required atmosphere. Full details are given in the original paper, to which the reader is referred.

Using this technique and employing *n*-butyl alcohol-acetic acid-water mixture as solvent we investigated the behaviour of ergot alkaloids on Whatman No. 1 paper. The alkaloids gave little or no colour with the ninhydrin reagent but their positions on the paper were readily detected by their fluorescence in ultra-violet light. It became immediately evident

that the water-insoluble alkaloids, of the ergotoxine and ergotamine groups, passed down the paper with the solvent front. However, separation of the water-insoluble from the water-soluble alkaloids occurred and, what is more important, ergometrine and ergometrinine passed down the paper at different rates and consequently could be separated and identified. The solvent employed was prepared by shaking together *n*-butyl alcohol (4 vols.), glacial acetic acid (1 vol.) and water (5 vols.). After standing the upper layer of *n*-butyl alcohol was placed in the trough and used as the moving phase on the chromatogram, while the lower aqueous acetic acid layer was placed in a dish at the bottom of the drain-pipe. Satisfactory chromatograms resulted when the alkaloids were employed as tartrates, lactates or maleates, but when present as sulphates little movement on the paper took place. A chromatogram was prepared by placing 0·05 ml. of solution containing 5 to 10 μg. of alkaloid on the paper. After allowing the solvent to run down the paper for 12 to 18 hours the chromatogram was air dried and examined using a suitable source of filtered ultra-violet light. With samples of pure ergometrine and ergometrinine, in solution as maleates, the following R_F values were obtained:—Ergometrine, 0·59; ergometrinine, 0·68.



Left: Ergometrine. *Right*: Ergometrinine.
Centre: Mixture of Ergometrine and Ergometrinine.

Fig. 1. Section of chromatogram of ergot alkaloids in ultra-violet light, showing the relative positions of ergometrine and ergometrinine.

Figure 1 shows a typical chromatogram, photographed while exposed to ultra-violet light, and illustrates the application of the method for purposes of identification.

An obvious extension of this work is the development of a method for estimating ergometrine, the most important ergot alkaloid. In this we

have only been partially successful. The fluorescent spots corresponding to the respective alkaloids may be marked on the chromatogram with pencil and subsequently measured in area or cut out and extracted to remove the alkaloids. These methods did not yield satisfactory results, however, for the quantitative removal of the alkaloids from the paper proved surprisingly difficult and the area of the spots could not be used for quantitative work. Our most successful results were obtained by preparing a series of standard spots of ergometrine on a paper chromatogram at the same time as the sample under test was examined, and matching the fluorescence of the respective spots under ultra-violet light. As with most fluorescent methods difficulties caused by quenching of the fluorescence at high concentrations were encountered and it was necessary to use solutions containing less than 0·001 per cent. of ergometrine before the fluorescence approached a linear relationship to concentration. Matching was performed visually and the precision was not high, the experimental error being of the order of ±20 per cent. As an alternative procedure chromatograms were prepared using serial dilutions of the standard and test solutions until dilutions were reached at which the fluorescence of the alkaloidal spots disappeared. This technique failed owing to the difficulty of determining the end-point, for even at extreme dilutions the fluorescence persisted.

The Ergometrine Content of Ergot

The first attempt to determine chemically the water-soluble alkaloids in ergot was made by Hampshire and Page[4] and their method has formed the basis of the B.P. 1948 assay process, in which the water-soluble alkaloidal content is expressed in terms of ergometrine and estimated from the difference between estimations of the total and water-insoluble alkaloids. Numerous other researches on the same topic have been published but the only processes of note are those developed by Grove[5] and by Powell *et al.*[6] by which the ergometrine is extracted and determined directly by colorimetric assay. These latter processes have been further developed in a collaborative study described by Smith[7] and have also been included in a report on the assay of ergot issued by the American National Formulary Committee[8].

We have estimated the ergometrine content of ergot, using our chromatographic technique and, for this purpose, the following process was used. 5 g. of ergot, ground to No. 60 powder, was defatted by extraction with light petroleum and air dried at room temperature. The resulting powder was thoroughly mixed with 0·3 g. of sodium bicarbonate and water was added, drop by drop, with stirring until there was obtained a well damped mass, which was then placed in a percolator (made from a piece of glass tubing 1 inch in diameter) and extracted with peroxide-free ether containing 5 per cent. of alcohol. Extraction of the alkaloids was slow and was best performed by drawing off 10 ml. of percolate at hourly intervals until about 70 ml. had been collected, after which the marc and solvent were allowed to remain in contact overnight before further percolate was withdrawn. Collection of the percolate was then continued, as described above, until another 100 ml. had been withdrawn, when the

process was stopped and the marc allowed to remain in contact with the solvent overnight. The extraction was completed in the morning by drawing off portions of percolate at half-hourly intervals until the total volume of extract amounted to 200 to 250 ml. The percolate was collected in an amber glass bottle and the whole process carried out in a dark room. After transferring the ethereal extract to a separating funnel the alkaloids were removed by shaking with 6 quantities, each of 10 ml., of 5 per cent. lactic acid; the acid extracts being collected in a graduated cylinder and the volume adjusted to 100 ml. with distilled water. Portions of this extract were then suitably diluted with 1 per cent. lactic acid until 0·05 ml. placed on a No. 1 Whatman paper strip and developed with *n*-butyl alcohol-acetic acid-water mixture, as described in the first section, gave a fluorescent spot approximately equal in intensity to that obtained with an ergometrine standard containing 0·2 to 0·5 μg. of ergometrine in 0·05 ml. By running a series of standards on the same paper the ergometrine content of the ergot was estimated.

Table I summarises the results obtained on samples of ergot, which were also assayed by the process of the B.P. 1948 and by that of the American National Formulary Committee[8].

It will be seen that the N.F. and chromatographic methods, in most cases, gave results for the ergometrine contents which were in reasonable agreement, but that the B.P. process afforded figures for the water-soluble alkaloids far in excess of the ergometrine present. Accordingly some of the final tartaric acid extract containing the total alkaloids, obtained by the B.P. process, was submitted to chromatographic analysis. Besides alkaloids of the ergotoxine group ergometrine and ergometrinine were detected. In addition a slower moving band was present above the ergometrine and this we were able to identify as being due to lysergic and *iso*-lysergic acids. It thus became clear that the use of boiling ether for extraction resulted in partial hydrolysis of the alkaloids with the production of lysergic acid, which was removed together with the water-soluble alkaloids and was estimated as ergometrine. The B.P. process therefore, did not yield reliable figures for the ergometrine content of the drug.

TABLE I

Sample of Ergot	B.P. 1948 Process		National Formulary Committee Process		Ergometrine determined chromatographically
	Total alkaloids expressed as ergotoxine	Water-soluble alkaloids expressed as ergometrine	Total alkaloids expressed as ergotoxine	Water-soluble alkaloids expressed as ergometrine	
	per cent.	per cent.	per cent.	per cent.	per cent.
1	0·22	0·043	0·215	0·023	0·019
2	0·10	0·0125	0·12	0·0086	0·006
3	0·19	0·038	0·195	0·025	0·026
4	0·16	0·040	0·195	0·020	0·016
5	0·20	0·045	0·21	0·022	0·024

Some preliminary experiments with liquid extract of ergot B.P. 1914 and liquid extract of ergot B.P. 1932 showed that the presence of ergometrine in these preparations could readily be confirmed by suitably

diluting with 1 per cent. lactic acid and preparing a chromatogram. Rough assays could be carried out as the colouring matter of the extracts remained almost stationary at the top of the chromatograms. Old extracts contained both ergometrinine and lysergic acid.

Ergometrine Preparations

At the British Pharmaceutical Conference 1948 Foster and Stewart[9] gave an account of the stability of ergometrine preparations. During the discussion on the paper Eastland[10] questioned the conclusion of the authors that the drop in biological potency of ergometrine maleate injection on storage was due to conversion of ergometrine into ergometrinine, and stated that this was at least partially due to hydrolysis of ergometrine to lysergic acid. Eastland supported his views by experimental data showing that while colorimetric assays of ergometrine injection, filled into ampoules under nitrogen and incubated for some months at 45°C., showed little loss of alkaloid by direct assay, a lower figure for the alkaloidal content was obtained if the injection were rendered alkaline and the alkaloid extracted before being estimated. It was suggested that the difference was due to lysergic acid.

During the past year we have carried out direct and indirect assays on ampoules of injection of ergometrine maleate B.P. 1948 which had been stored at room temperature for periods up to 10 years. For indirect assays the alkaloid was extracted with ether after making alkaline and saturating the solution with sodium chloride as described by the N.F. Committee[8]. Preliminary extraction experiments using solutions of pure ergometrine and ergometrinine maleates, of the same strength as used for the injection, showed that 90 to 95 per cent. recovery of ergometrine and 95 to 100 per cent. recovery of ergometrinine resulted. When applied to ergometrine maleate injection the process gave a recovery of 90 to 95 per cent. of the total alkaloidal content when freshly prepared ampoules were employed, but with 5-year-old ampoules the recovery was only 75 to 80 per cent. Allowing for a 10 per cent. loss during the extraction it would appear that some 15 per cent. of alkaloid in the older ampoules remains to be accounted for. The results of Eastland were therefore confirmed.

In order to study the composition of the injection more closely

TABLE II

Sample of ergometrine maleate injection B.P. 1948	Time of storage at room temperature	Components identified on chromatogram
Freshly made and unsterilised ...	—	Ergometrine
Freshly made and sterilised at 10 lb. pressure of steam for 30 minutes	—	Ergometrine Ergometrinine Traces of lysergic and *iso*-lysergic acids
Sterilised and stored at room temperature	5 years	Ergometrine Ergometrinine Lysergic and *iso*-lysergic acids

samples were examined by paper partition chromatography using *n*-butyl alcohol-acetic acid-water mixture as solvent. The results are summarised in Table II.

The general results indicated that on sterilisation of the injection some conversion, estimated to be approximately 20 per cent., of ergometrine to ergometrinine occurs. Very little further conversion appears to take place on storage at room temperature which, however, results in slow hydrolysis of the alkaloids with the formation of lysergic and *iso*-lysergic acids. The presence of the lysergic acids is better shown by using a basic solvent prepared by shaking a mixture of *n*-butyl alcohol (4 vols.), water (5 vols.) and pyridine A.R. (1 vol.), allowing to separate and using the *n*-butyl alcohol layer as the moving phase on the chromatogram, while the aqueous layer is used for saturating the atmosphere of the chamber. Under these conditions and using No. 1 Whatman paper the R_F values were as follows:—Lysergic acid, 0·2; *iso*-lysergic acid, 0·4.

The presence of lysergic acid was further confirmed by extracting the alkaloid from some old injection, the final alkaloidal extract being made with 1 per cent. lactic acid. On preparing a chromatogram with this extract ergometrine and ergometrinine were identified but the lysergic acids, present in the chromatogram of the original injection, had disappeared.

It was of interest to examine chromatographically tablets of ergometrine maleate which had been stored at room temperature and, for this purpose, the tablets were extracted with, or dissolved in, 1 per cent. lactic acid. Very little, if any, formation of ergometrinine or lysergic acid was detected in tablets which had been stored for periods up to 5 years.

Identification of Water-Insoluble Alkaloids

The water-insoluble alkaloids of ergot are distinguished from ergometrine by the presence in their molecular structures of certain amino-acids. Jacobs and Craig[11] found among the products of alkaline hydrolysis of ergotinine the lactam of a dipeptide, derived from L-phenylalanine and D-proline, while Smith and Timmis[12] showed that ergosine gave a similar lactam of the dipeptide of L-leucine and D-proline. Stoll, Hofmann and Becker[13] showed that ergocornine, ergocristine and ergokryptine, isolated from the ergotoxine group of alkaloids, also contained amino-acids of the L-series in addition to D-proline.

TABLE III

ALKALOIDS CHARACTERISED BY STRUCTURES DERIVED FROM LYSERGIC OR *iso*-LYSERGIC ACID, AMMONIA, A KETO ACID, D-PROLINE AND ONE OTHER AMINO-ACID

Additional amino acid	Ergotamine group (pyruvic acid group)	Ergotoxine group (Dimethyl-pyruvic acid group)
L-phenylalanine	Ergotamine Ergotaminine $C_{33}H_{35}O_5N_5$	Ergocristine Ergocristinine $C_{35}H_{39}O_5N_5$
L-leucine	Ergosine Ergosinine $C_{30}H_{37}O_5N_5$	Ergokryptine Ergokryptinine $C_{32}H_{41}O_5N_5$
L-valine	—	Ergocornine Ergocorninine $C_{31}H_{39}O_5N_5$

The relationships of the known water-insoluble alkaloids are summarised in Table III embodying data by Stoll *et al*[13].

The exact mode of linkage of the amino-acids in these alkaloids is not known but Stoll and Hofmann[14] have reported that thermal degradation of ergotamine yielded pyruvyl-phenylalanyl-proline, and Stoll[15] has further reported finding dimethylpyruvyl-valyl-proline, together with lysergic acid amide, in the products from the alkaline hydrolysis of ergocornine.

It is thus clear that the identification of the amino-acids obtained on acid hydrolysis would be of great assistance in the identification of pure alkaloids and, in the absence of major quantities of extraneous amino-acids, might be of help in the examination of cruder preparations. The occurrence of valine in ergocornine and ergocorninine is a specific test for this inter-convertible pair of alkaloids. The presence of phenylalanine would indicate ergotamine or ergocristine and their isomers, further tests such as the identification of the keto-acid and the determination of physical properties being necessary for complete identification of the alkaloids. The finding of leucine would be equally significant for the detection of ergosine and ergokryptine and their isomers.

We have identified the amino-acids in the acid hydrolysates of the alkaloids by the method of paper partition chromatography in the following manner. 10 mg. of the alkaloidal preparation together with 1 to 2 ml. of concentrated hydrochloric acid was heated in a sealed tube at 100°C. for 16 hours. After cooling, the contents of the tube were transferred to an open dish and evaporated to dryness on a steam bath. The dark residue was extracted with 0·2 ml. of distilled water and, without separating the insoluble matter 0·01 ml. of the suspension was placed on the paper. The chromatogram was prepared on Whatman No. 4 paper using *n*-butyl alcohol-acetic acid-water mixture. Although the positions of the amino-acids on the well-dried paper were revealed by their bluish-white fluorescence in filtered ultra-violet light, a more specific test in the presence of fluorescent alkaloids was afforded by the ninhydrin reaction, carried out by spraying the paper with 0·1 per cent. ninhydrin in equal parts of *n*-butyl alcohol and chloroform, drying and then developing by heating in an oven at 100°C. The amino-acids were identified by their R_F values and their ninhydrin colour reactions, leucine and valine yielding reddish purple spots while those due to phenylalanine and proline were grey-blue and yellow respectively. Under the above conditions hydro-

TABLE IV

Alkaloid	Amino-acids, identified chromatographically
Ergotamine	Proline, phenylalanine
Ergotaminine...	Proline, phenylalanine
Ergocristine	Proline, phenylalanine and faint trace of valine
Ergocristinine (ergotinine)	Proline, phenylalanine and faint trace of valine
Ergocornine (ergotoxine)	Proline, valine and trace of phenylalanine

lysis of ergine, the amide of lysergic acid, gave a continuous streak showing a variety of colours, but no major spots reacting with ninhydrin. A similar streak often appeared on chromatograms prepared with hydrolysates of water-insoluble alkaloids but was fainter and caused no difficulty in the identification of the amino-acids.

Using the above technique the results shown in Table IV were obtained with samples of " pure " alkaloids.

From these preliminary experiments it is obvious that not only is paper chromatography a valuable tool for identifying but also for assessing the purity of ergot alkaloids. For example, it is clear that in the cases of the ergocristine and ergocornine examined each alkaloid was contaminated with traces of the other. When assessing the degree of contamination it is necessary to remember that phenylalanine, under the conditions employed, gives less colour than leucine or valine and one of the quantitative applications of paper chromatography should be applied.

Other minor ninhydrin spots have been noted in the chromatograms, but at present no assignment of these to any known constituent of the hydrolysate can be made. Alkaline hydrolysates have not shown the presence of amino-acids but, in general, give two elongated spots reacting with ninhydrin. The substances in these spots have yet to be identified.

Discussion

It was stated early in this paper that available methods of ergot assay lacked specificity and it is felt that the application of paper partition chromatography, now described, has done something to remedy this deficiency.

The need for a specific identification test for ergometrine has long been felt by workers in this field and it is a matter of great importance to the chemical manufacturer who wishes to purchase ergot for the manufacture of ergometrine. By paper chromatography it is a simple matter, even with a small sample of drug, to state whether ergometrine is present and approximately in what quantity. The application to liquid extracts of ergot has also been described.

Of all ergot preparations perhaps injection of ergometrine maleate is the most important. As a result of the present work a much clearer picture of the changes which occur during the manufacture and storage of the injection has been obtained and, in view of the very small quantity of injection required for a test, the chromatographic technique has opened the field for much fuller investigation. It has been seen that conversion of ergometrine to ergometrinine occurs on heat sterilisation of the injection and it may be that sterilisation at room temperature by candle filtration might afford a better preparation. The *p*H of the injection may influence the alkaloidal equilibrium and we have experiments in progress to explore this aspect of the problem. Hydrolysis of the alkaloid in the injection has been confirmed and this factor must be added to the previously accepted causes of deterioration[9]. It is considered unlikely that the hydrolysis of ergometrine in solution can be avoided.

The close relationship of the ergot alkaloids to the polypeptides has been emphasised by the use made of chromatograms, prepared from the

hydrolysates of the alkaloids. In spite of the amount of work so far done on the ergotoxine group of alkaloids it seems doubtful whether complete separation of the individual alkaloids has yet been achieved and, in this connection, it is certain that the chromatograms of the amino-acids will be of great value in testing highly purified specimens for traces of other alkaloids. A long-standing controversy as to whether ergotamine and ergotoxine ever occur together in the same ergot[16] has not been resolved and chromatography may well have something to contribute in this field. Preliminary results with chromatograms, prepared from hydrolysates of single sclerotium of *Claviceps purpurea* have indicated that the amino-acids found are a good indication of the alkaloids present in the drug, about which there is still much to be learnt.

Summary

1. A study has been made of the application of paper partition chromatography in the ergot field.

2. A technique for the separation and identification of ergometrine and ergometrinine, when present in mixtures of total ergot alkaloids, has been described.

3. The method has been extended so that approximately quantitative results may be obtained and, in this way, the ergometrine contents of samples of ergot have been estimated.

4. The changes which take place in injection of ergometrine maleate B.P. 1948, during manufacture and storage have been studied chromatographically. It was found that during heat sterilisation some conversion of ergometrine to ergometrinine occurs. On storage some hydrolysis giving rise to lysergic and *iso*-lysergic acids takes place.

5. By preparing chromatograms from the hydrolysates of water-insoluble ergot alkaloids, identification tests for individual alkaloids have been developed.

We wish to thank Mr. H. M. Hood, B.Sc., for the photograph of a chromatogram, Mr. R. L. Grant, M.Sc., for a sample of ergometrinine and Dr. S. Smith for specimens of lysergic and *iso*-lysergric acids. We are also indebted to the Directors of The Wellcome Foundation for permission to publish this paper.

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DISCUSSION

The paper was read by Dr. G. E. Foster.

The CHAIRMAN said that he would particularly like to congratulate the authors on the ingenious method of hydrolysing the alkaloids and separating the elements chromatographically. At last year's Conference he had suggested that the colour test was not really an indication of the amount of deterioration. The work reported in this paper confirmed that and gave valuable new methods of determining the amount of deterioration. He hoped some agreement would be reached on the names for ergot alkaloids.

DR. F. HARTLEY (London) said that preliminary experiments carried out by his colleagues with a similar object to that of Dr. Foster had shown interesting results but they had not been able to pursue them to the extent that Dr. Foster had done. In examining different solvents for the separation of ergot alkaloids on a chromatogram, they had found that *iso*butyric acid gave $R_F 0{\cdot}90$. *n*-Butanol-acetic acid gave an R_F value of 0·55 which agreed well with the figure 0·59 given by Dr. Foster.

In addition to the amino acids obtained by the hydrolysis of ergometrine, 2-amino-propanol was produced. This was readily distinguishable from the amino acids by the ninhydrin reaction and in their hands, using *n*-butanol-acetic acid it had an R_F value of 0·33. This observation might assist in studying the deterioration of ergometrine injection and perhaps Dr. Foster could determine the 2-amino-propanol in his five years old sample.

PROFESSOR BRINDLE (Manchester) said that they had been trying chromatography for the estimation of ergot alkaloids and had found difficulty in extracting the alkaloids from the chromatogram. They had tried to extract the ergotoxine and ergometrine (quantitatively) by prolonged extraction in a continuous extractor with ether, but on examination of the chromatograms in ultra-violet light, a fluorescence persisted. They had had the same difficulty in extracting the alkaloids from silica gel. They had achieved more success using kieselguhr and a citrate-phosphate buffer at *p*H5. The ergotoxine had been recovered quantitatively, but the ergometrine was not so easy. In a deteriorated solution of ergotoxine they had found a difference between the colorimetric assay, done directly on the solution, and that performed on the extracted alkaloids. They were satisfied it was due to lysergic acid, which did not affect the colour test if the alkaloids were extracted first. He had noticed that deterioration varied according to *p*H. With a solution at *p*H3 there was good agreement between the colorimetric and biological assays, with the biological result a little below the colorimetric. If deterioration occurred at *p*H5 or over there was a big difference between the two assays, with the biological result about half that of the colorimetric assay. Had Dr. Foster any observations to make on the considerable difference in the type of deterioration according to *p*H?

DR. W. MITCHELL (London) asked if the colours of the ultra-violet fluorescence were distinctive for ergometrine and ergometrinine. Dr. Foster had said that the accuracy of the method of comparing the intensity of

fluorescence was about ± 20 per cent. That was not a very high accuracy. Was it also necessary to make allowance for the size of the spot? If the standard spot was smaller than the test spot, presumably it would have some effect on the intensity. Was there any possibility of alteration of the ergometrine due to the somewhat prolonged exposure to air? This method for the testing of ergot would be useful, but the limiting factor was the time involved since it appeared to require at least five working days.

DR. R. E. STUCKEY (London) said that in his laboratory they had had some experience in removing amino-acids from chromatograms for their quantitative estimation. Was Dr. Foster sure that the residual fluorescence was due to traces of ergometrine remaining in the paper and not to peptisation of the paper fibres which was liable to occur? He would like to suggest that the chromatogram be run not as a spot but as a band with ten or more spots. This would increase the amount of ergometrine making a chemical or spectrophotometric estimation possible and the amount of ergometrine left in the paper might then be low in comparison with that extracted. Was any information available on that point?

DR. G. E. FOSTER (Dartford) said he was very interested to learn about Dr. Hartley's experience with other solvents. In view of Dr. Stuckey's suggestion they might have to look into the problem of extraction more closely, but so far they had found it impossible to remove the fluorescence completely. As to the difference of *p*H, they had prepared an ergometrine maleate injection, and adjusted the *p*H to various values from 3 to 6 by the addition of maleic acid. The chromatograms were obtained after sterilisation, all the spots being put on one paper. As the *p*H decreased, the amount of ergometrine gradually increased until at *p*H3 or 3·5 there had been very little ergometrinine there at all, and the amount of lysergic acid seemed to decrease at the same time.

A recent sample of ergometrine maleate injection from the U.S. had been found to contain very little ergometrinine, but the acidity had been much higher than that of the B.P. injection.

The colour of the fluorescence was the same for ergometrine and ergometrinine. The 20 per cent. error of visual comparison of the fluorescence was within the limits usual in this kind of work. The size of the spots did not seem to be important. Four of the five days required for the method were taken up with percolation. The ratio of total alkaloids to ergometrine was more or less constant in Spanish or Portuguese ergot, and by doing a rough chromatograph extraction it was possible to obtain a quick qualitative result overnight.

Conversion of the alkaloids on the paper did not appear to occur. They had no information on peptisation of the paper fibres. Neither had they done any tests using a band technique.

PROFESSOR H. BRINDLE (Manchester), in answer to Dr. R. E. Stuckey said they had tried increasing the amount of the alkaloids used in paper chromatography. They could not get a quantitative recovery from the band. As they increased the band, the amount of paper was increased, and it was adsorption on the paper which caused the difficulty.