A few tests were made on the commercial oleoresins of ginger. Four samples were tested and found to produce a threshold pungency in concentrations of 5 mg. per L.

CONCLUSIONS.

1. A modified method for the bioassay of capsicum has been developed and proved successful in a series of assays by our students.
2. As standards of pungency it is recommended that 20 mg. of capsicum per L., and 3.5 mg. of oleoresin of capsicum per L. should produce the same degree of pungency as 16 mg. of piperine per L. (Threshold concentrations.)
3. This method is suitable for the bioassay of ginger and its oleoresin.
4. As standards of pungency threshold concentrations of 400 mg. of ginger and 5 mg. of oleoresin of ginger per L. are suggested.

BIBLIOGRAPHY.

(1) G. Cohn, "Die organischen geschmacksstoffe," 1914.
(2) A. G. DuMez, "A century of the United States Pharmacopœia, 1820–1920, I. The Galenical Oleoresins," *Inaug. Dis.*, Wisc., 1917.
(3) E. N. Gathercoal and R. E. Terry, "The capsicum monograph in U. S. P. X," JOUR. A. PH. A., 10 (1921), 423–428.
(4) James C. Munch, "Bioassays of Capsicum and Chillies. I," *Ibid.*, 18 (1929), 1236–1246.
(5) James C. Munch, "The Bioassay of Capsicum, U. S. P. X," *J. A. O. A. C.*, 13 (1930), 383–385.
(6) James C. Munch, "Bioassays, A Handbook of Quantitative Pharmacology," 1931.
(7) W. L. Scoville, "Note on Capsicums," JOUR. A. PH. A., 1 (1912), 453–454.
(8) P. Valenzuela, "Philippine Ginger," *Ibid.*, 15 (1926), 652–661, 734–744.
(9) R. Wasicky and F. Klein, "Über die wertbestimmung von capsicum," *Festschrift für A. Tschirch* (1926), 357–361.
(10) E. H. Wirth and E. N. Gathercoal, "Report of the Scoville Organoleptic Method for the Valuation of Capsicum," JOUR. A. PH. A., 13 (1924), 217–219.

NOTES ON THE B. P. COLORIMETER TEST FOR ERGOT.*

BY F. A. UPSHER SMITH.

The color test for ergot devised by Maurice I. Smith (1) in 1930 soon found favor in Great Britain, so that it was adopted in the 1932 edition of the British Pharmacopœia.

Wokes (2) found that the test fails to distinguish between the inactive and active constituents of ergot. The four alkaloids, ergotoxine, ergotamine, ergotinine and ergotaminine, all gave the identical blue color with the Maurice Smith reagent when examined spectroscopically. In spite of the fact that this test does not measure solely the physiologically active substances in ergot, it was adopted for the following reason: The colorimeter test measures the total alkaloid with greater accuracy than the biological method measures the physiologically active alkaloid.

In the Report of the Sub-Committee on Ergot of the British Pharmacopœia Revision Committee (3), the conclusion was reached that the results by the bio-

* Scientific Section, A. PH. A., Madison meeting, 1933.

logical test are lower than those given by the chemical methods; the ratio of the average results is 2 : 1. In setting the potency of ergot at "0.05% of the total alkaloids of Ergot, calculated as ergotoxine," the committee took into consideration the evidence which suggests that 60 to 70% of the total alkaloid consists of ergotoxine. Consequently, a total alkaloidal percentage of 0.05 is equivalent to an ergotoxine percentage of 0.03.

Up to the present time the majority of authorities have regarded either ergotoxine or ergotamine as the physiologically active constituent of ergot. The U. S. P. Cock's Comb method, the Broom-Clark epinephrin-reversal method, and the Maurice Smith colorimeter test all agree in valuing the ergot or its preparations according to the amount of alkaloid present. With this idea in mind, the fluidextracts of the U. S. P. and of the B. P. have been made with acidified diluted alcohol, in order to ensure as high an alkaloidal content as possible. The aqueous preparations of ergot, known to be deficient in alkaloid, have accordingly fallen into disrepute, in spite of the fact that the introduction of ergot into medicine was in the form of an infusion or decoction of the drug.

The consensus of opinion on ergot might, therefore, be held to favor the use of preparations containing the correct proportion of alkaloids, such as the fluidextracts of the U. S. P. and of the B. P., and to warrant the discarding of aqueous preparations of ergot, deficient in alkaloids. Strange to say, the situation has been complicated by the publication in June of last year of a paper by Dr. Chassar Moir (4), Obstetric Unit, University College Hospital, London, which has once more reopened the whole question as to what is the active principle of ergot. Working on human patients, Dr. Moir found that the effect of ergot when taken by mouth was to induce with remarkable rapidity strong uterine contractions, which lasted only for a limited time. This was in contrast with the known effect of ergotoxine, which is slower to start and more persistent in duration. Moir concluded that there must be in ergot some principle not previously isolated and identified. Dr. Moir found that the aqueous extracts and fluid preparations of ergot, even when old and containing at best but a trace of alkaloid, possessed the activity ascribed to this unknown substance. He found, however, that the B. P. liquid extract of ergot, which agrees closely in formula and composition with the U. S. P. fluidextract of ergot, also possessed this effect in about the same degree. It follows, therefore, that ergot contains an unknown active principle with a rapid but fleeting action, and alkaloids, such as ergotoxine, which are slower in action but more persistent. It is possible that the unknown principle initiates the contraction of the uterus, and that in turn the ergotoxine (or the ergotamine) carries on the contraction for a longer time. The search for Dr. Moir's unnamed active principle will be followed with interest. If its presence is confirmed, then the determination of the amount of alkaloid in ergot does not completely evaluate the drug.

Several investigators have published results of their work on the colorimeter test, including M. I. Smith and Stohlman (5), Swanson, Powell, Stevens and Stuart (6), Powell, Schulze and Swanson (7), Wokes (8), Swoap, Cartland and Hart (9), J. A. C. van Pinxteren (10), Allport and Cocking (11), Gerlough (12), Lozinski, Holden and Diver (13) and (14), and Smelt (15).

The author has tried the B. P. colorimeter test on several samples of ergot,

and compared results with those obtained by the Allport and Cocking modification. In the B. P. test as devised by M. I. Smith, the standard consists of 1 cc. of ergotoxine ethanesulphonate solution in water (containing 0.1 mg. of anhydrous ergotoxine) to which is added 2 cc. of a 1 in 800 solution of dimethylaminobenzaldehyde in 50% sulphuric acid v/v. The resulting blue colors are matched in a colorimeter. If the colors match, the sample contains 0.4% of alkaloids, calculated as ergotoxine.

The B. P. method of extraction consists in taking 12 Gm. of ergot in powder, defatting, and shaking the defatted powder with 120 cc. of ether for 10 minutes. Then add 0.5 Gm. of light magnesium oxide diffused in 20 cc. of water, and shake at intervals during 30 minutes. Now add 1.5 Gm. of powdered tragacanth, shake vigorously, filter through cotton, and transfer 100 cc., representing 10 Gm. of ergot, to a separator. The ethereal solution of the alkaloids is shaken with several portions of a 1% solution of tartaric acid, the ether removed by gentle heat and made up to 40 cc. One cc. of this solution represents 0.25 Gm. of ergot. When the B. P. method is followed on a hot day, there is difficulty in separating 100 cc. of ethereal solution.

Recently, Miss E. M. Smelt (15) has suggested a modification of the B. P. method of testing the fluidextract in order to prevent emulsification during the shaking with ether:

Five cc. of the fluidextract are diluted with 25 cc. of distilled water, rendered slightly alkaline with 10% ammonia solution, and extracted with successive portions of 40, 35, 30 and 30 cc. of ether. The first portion of ether should not be shaken too vigorously, but less care is needed in shaking the subsequent portions. The ethereal solution is washed as directed in the B. P., and then extracted five times with successive portions of 4 cc. (or for strong liquids, 5 cc.) of a 1% solution of tartaric acid. After removal of ether, the solution is adjusted to 20 cc., or for strong fluidextracts, to 25 cc.

Allport and Cocking have insisted on the use of pure anesthetic ether, to avoid oxidation of the alkaloids.

The Allport-Cocking modification consists simply in adding a small amount of ferric chloride or ferric sulphate to the reagent. Using a 10% solution of ferric chloride, 5 cc. are diluted to 100 cc. with distilled water. The reagent is then mixed as follows:

Dimethylaminobenzaldehyde	0.125	Gm.
Sulphuric Acid, U. S. P.	65	cc.
Diluted Ferric Chloride solution	1	cc.
Distilled water to make	100	cc.

The advantage of this reagent consists in the full development of the color in about five minutes, even in the dark, so that prolonged exposure to light is unnecessary. In following the B. P. test, the solution, after adding the reagent, is warmed to 45° before exposure to light, but this is not necessary in using the Allport-Cocking reagent, as the temperature rises to about 45° on mixing the liquids. The solution should stand for at least 5 minutes before comparing the colors, in order to allow ample time for the color to develop. The following results were obtained with six samples of ergot, against a fresh solution of ergotoxine ethanesulphonate:

Sample.	B. P. Reagent.	Allport-Cocking Reagent.
1	0.170	0.171
2	0.210	0.191
3	0.082	0.073
4	0.100	0.090
5	0.145	0.134
6	0.236	0.222

(Results in Gm. total alkaloids in 100-Gm. ergot.)

The use of ergotoxine ethanesulphonate as a standard raises the question as to whether that standard is uniform from time to time. It seemed to be desirable to try to match this standard against a colored chemical solution. For this purpose an ammoniacal solution of cupric sulphate in distilled water was prepared. After numerous trials, it was found that a solution containing 0.67% of C.P. crystalline cupric sulphate or the equivalent amount of the desiccated salt matched a freshly prepared solution of ergotoxine ethanesulphonate containing 0.1 mg. of anhydrous ergotoxine in 1 cc. The similarity in color of the copper solution to the ergot solution treated with the B. P. reagent or the Allport-Cocking modification, is remarkably close. It is best to match the colors in daylight when using a Bausch and Lomb Duboscq colorimeter. The probable value of the use of a copper solution as a super-standard is seen in the following results. In one set of experiments the same ergot samples were matched (*a*) against a freshly made B. P. ergotoxine solution treated with the B. P. reagent; and (*b*) against an ammoniacal copper sulphate solution containing 0.67% of C.P. crystalline cupric sulphate:

Samples.	B. P. Ergotoxine Solution and B. P. Reagent.	Ammoniacal Cupric Sulphate Solution.
1	0.170	0.162
2	0.210	0.168
3	0.082	0.074
4	0.100	0.080
5	0.145	0.126
6	0.236	0.199

Another set of experiments was made, replacing the B. P. reagent by the Allport-Cocking modification, using the same samples of ergot.

Samples.	B. P. Ergotoxine Solution and Allport-Cocking Reagent.	Ammoniacal Cupric Sulphate Solution.
1	0.171	0.162
2	0.191	0.190
3	0.073	0.078
4	0.090	0.099
5	0.134	0.126
6	0.222	0.216

On the whole, these two sets of experiments show a fairly close agreement and suggest that the copper standard may have a useful function.

It would be a distinct advantage if we could check up our ergotoxine standard against such a fixed standard, easily obtainable. It would guard against using an ergotoxine salt or a solution of the same that had deteriorated. The long-continued heat has delayed some of the experimental work that was planned, due to the

difficulty of handling the ether solutions on an aliquot basis. These preliminary observations as to the use of copper sulphate as a check on the standard are offered in the hope that others may try it, and possibly improve on it.

Recently, R. Freudweiler (16) has suggested the use of a 2% solution of vanillin in concentrated sulphuric acid (free from nitric acid) in place of the B. P. reagent. When 2 cc. of the vanillin reagent are added gradually to 1 cc. of an aqueous solution of the alkaloids of ergot, a purple color develops, which is stable for hours, and under the given experimental conditions, its intensity is proportional to the amount of alkaloid present. Equimolecular proportions of ergotoxine, ergotamine and ergotinine are stated to give equal intensities of color. Allport and Cocking (11) tried vanillin (and other aldehydes) in place of dimethylaminobenzaldehyde, but found none of them developed a color without exposure to bright light. In trying out this reagent it was found that the temperature ran up quickly to 93° and in one case to 98°. On adding the reagent to the ergotoxine solution surrounded by cold water, a temperature of 72° was reached. A wine-red color was developed.

A solution was then made of 2% vanillin in 65% v/v of concentrated sulphuric acid. The color when fresh was bright golden yellow, but by the next day the bright gold effect had given way to a dull yellow. On adding this solution to the ergotoxine, the temperature rose to 45–52°, without using precautions to keep the solution cool. This temperature is not considered too high for the alkaloids. The color of the solution was a beautiful purple. But it was found a difficult color to match in the Duboscq colorimeter, either by daylight or artificial light, showing rose-red, and lacking the bluish or purplish tint of the ergotoxine solution. The following figures give the results with the same samples of ergot as before, tested against a fresh solution of ergotoxine with the same reagent:

Sample.	2% Vanillin in 65% Sulphuric Acid v/v.
1	0.148
2	0.167
3	0.069
4	Not tested
5	0.109
6	0.174

Combining all results into one table, the following figures make it easy to compare results:

Sample.	Sample and Ergotoxine Sol. Treated with B. P. Reagent.	Sample Treated with B. P. Reagent Compared with Cu Solution as Standard.	Sample and Ergotoxine Sol. Treated with Allport-Cocking Reagent.	Sample Treated with Allport-Cocking Reagent Compared with Cu Solution as Standard.	Sample and B. P. Ergotoxin Solution Treated with 2% Vanillin in 65% H_2SO_4 v/v.
1	0.170	0.162	0.171	0.162	0.148
2	0.210	0.168	0.191	0.190	0.167
3	0.082	0.074	0.073	0.078	0.069
4	0.100	0.080	0.090	0.099	Not tested
5	0.145	0.126	0.134	0.126	0.109
6	0.236	0.199	0.222	0.216	0.174

(Figures represent Gm. of total alkaloids in 100-Gm. ergot.)

In so far as I have used these reagents, I much prefer the Allport-Cocking reagent to vanillin. The colors are easier to match, and this reagent has given satisfaction in the hands of many workers in several countries.

It is to be hoped that the continued use of the Maurice I. Smith colorimetric method, as modified by Allport and Cocking, will prove to be a trustworthy guide as to the potency of ergot since it affords a simple means of checking biologic assays. There is need of further work on the relative values of results obtained by chemical and biologic methods of assay now in use. The papers published by Lozinski, Holden and Diver (13) and (14) are a step in the right direction.

BIBLIOGRAPHY.

(1) Maurice I. Smith, Reprint No. 1390, *Public Health Reports.*
(2) Frank Wokes, *Pharm. J.*, 128 (1932), 206.
(3) *Ibid.*, 127 (1931), 482.
(4) Chassar Moir, *Brit. Med. J.*, Part 1 (June 18, 1932), 1119.
(5) Smith and Stohlman, *J. Pharmacol. & Exper. Therap.*, 43 (December 1931), 621.
(6) Swanson, Powell, Stevens and Stuart, Jour. A. Ph. A., 21 (1932), 229.
(7) Powell, Schulze and Swanson, *Ibid.*, 21 (1932), 1003.
(8) Frank Wokes, *Quart. J. Pharm.*, 4 (1931), 420.
(9) Swoap, Cartland and Hart, Jour. A. Ph. A., 22 (1933), 8.
(10) J. A. C. van Pinxteren, *Pharm. Weekbl.*, 68 (1931), 688.
(11) Allport and Cocking, *Quart. J. Pharm.*, 5 (1932), 341.
(12) Gerlough, *Am. J. Pharm.*, 103 (1931), 644.
(13) Lozinski, Holden and Diver, *J. Pharmacol. & Exper. Therap.*, 42 (1931), 123.
(14) Same authors, *Pharm. J.*, 130 (1933), 137.
(15) E. M. Smelt, *Ibid.*, page 137.
(16) R. Freudweiler, *Pharm. Acta Helv.*, 7 (1932), 116, 139; through *Quart. J. Pharm.*, 6 (1933), 268.

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YEAST EXTRACT IN PILL MAKING.

Various yeast extracts are found to be suitable for pill making. Ext. faecis sicc. (D.A.B. VI) is used in conjunction with glycerinated water in pil. arsen. (0.001 Gm.), pil. creosoti (0.025 mil.), and pil. ferri redact. (0.05 Gm.). If the proportion of the powdered ingredients becomes too large for a satisfactory pill-mass to be made with the dry extract, a small quantity of ext. faec. spiss. (D.A.B. VI) may be added. For this purpose a mixture of equal weights of yeast extract, glycerin and water is kept ready and used as required; in this way the binding power of the dry extract is increased and a higher proportion of powders may be incorporated. Examples include quinine hydrochloride and salol with equal weights of dry and moist extract of yeast. The dry extract is suitable for preparing pills of ethereal oils, balsams, etc., and the pills are more soluble than when prepared with lanolin and kaolin, etc. Ext. faec. spiss. or powdered yeast dried at 100° C. may be used with the dry extract. The powdered yeast is superior to liquorice powder as a binding agent, and pills made with it more readily disintegrate, owing to absorption of moisture with consequent swelling. Examples quoted are for turpentine ((0.25 Gm.), santal oil (0.5 Gm.), and balsam of copaiba (0.5 Gm.), with 4 Gm. of dry extract and 8 Gm. of powdered yeast for 100 pills. If the pill formula already contains a soft extract, *e. g.*, extract of gentian, the yeast extract may be omitted.—R. Ruf (*Pharm. Weekbl.*, 33 (1933), 880).—Through *Pharmaceutical Journal.*