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The Biologic and Colorimetric Assay of Ergonovine in Ergot and Its Fluid Extract*

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Ergonovine is the new water-soluble base of ergot, the existence of which was first suspected by Moir (1) and clearly demonstrated by Davis, Adair, Rogers, Kharasch and Legault (2). This alkaloid, different from that of the water-insoluble members, ergotoxine and ergotamine, has a prompt oxytocic action when given by mouth as shown by Swanson and Hargreaves (3) in postpartum dogs. Ergonovine was isolated in a pure form, independently, by Kharasch and Legault (4), Dudley and Moir (5), Thompson (6) and Stoll and Burkhardt (7). The pharmacological action of the base was reported by Davis, Adair, Chen and Swanson (8), Chen, Swanson, Kleiderer and Clowes (9) and Brown and Dale (10). Ergonovine is now extensively used in medical practice as a uterine stimulant.

The present paper is concerned with the chemical separation and physiological assay of ergonovine in the crude ergot drug and fluid extract of ergot U. S. P. Previously, Swanson, Hargreaves and Chen (11) compared the various assay methods of pure ergonovine, and concluded that, in all respects, the isolated rabbit's uterus method gave most reliable figures. The oxytocic action of the isolated rabbit's uterus typifies ergonovine and eliminates the ergotoxine-like alkaloids, and the prolonged rhythmic contractions distinguish it from histamine and tyramine, both of which cause brief responses. The contractions of the mature virgin organ (isolated rabbit uterus) are proportional to the amounts supplied, which fact may be utilized for the quantitative evaluation of the base (ergonovine), just as the isolated guinea-pig uterus has been used for the assay of posterior pituitary extracts. This method (isolated rabbit's uterus), however, is of little value if the ergonovine is contaminated with the water-insoluble alkaloids (ergotoxine-like). In previous communications (8, 11), it has already been emphasized that the effects of ergonovine on the isolated rabbit's intestine and uterus are diminished or inhibited after the previous application of ergotamine or ergotoxine.

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Thus, because of this antagonism between ergonovine and ergotoxine, the Broom-Clark Method and the U. S. P. cock's comb Method are useless in differentiating the mixed alkaloids present in the crude ergot or its galenical preparation, the fluid extract U. S. P.

Hampshire and Page (12) described an assay process for ergot in which the water-soluble alkaloids are separated from the water-insoluble ones by shaking an ethereal extract with water and then determined colorimetrically. Schou and Bennekou (13) reported the distribution of ergonovine and ergotoxine between water and ether. Daglish and Wokes (14) have questioned the reliability of the Hampshire and Page method because of the difficulty of eliminating emulsions which appear when the ether extract is shaken with alkaline water. These authors found that acetone when used in extracting the drug in a mechanical shaker was a better solvent, without the formation of emulsions. In our hands, the water-soluble fraction (ergonovine) by the Hampshire and Page method could not be assayed biologically (isolated rabbit's uterus), because the solution still contained some of the water-insoluble alkaloids (ergotoxine-like). By further purification of this fraction (removal of the last traces of ergotoxine-like alkaloids), the colorimetric method still gave a much higher ergonovine content than the physiological method. This is probably due to the presence of the inactive water-soluble alkaloids (ergometrinine-like).

In view of the fact that Stoll (15), Kharasch and Legault (16), and we, in our own laboratory, found some samples of ergot completely devoid of ergonovine, and inasmuch as the known methods of assay are definitely unreliable, it became imperative either to modify the present methods or to devise a new one. One of the logical procedures, as outlined by Smith (17), would be the complete chemical separation of ergonovine from the ergotoxine-like alkaloids. This we have attempted to do. For the potency tests we have depended on the biological methods rather than the colorimetric method for reasons as previously stated. The ergonovine fraction was assayed by the isolated rabbit's uterus and the ergotoxine-like alkaloids were determined by the modified Broom-Clark Method (18). The physiologic test, however, was followed by the colorimetric method with the hope that an appropriate color filter could be found which would give readings similar to those of the biological assay. It was found that a light filter transmitting 6200 Å. gave the most concordant results.

EXPERIMENTAL

Method for Assaying the Crude Ergot Drug.—The directions for assaying the crude ergot drug may be given as follows:

"Place 12.5 Gm. of the defatted ground crude drug in a flask suitable for use in a mechanical shaker. Add 125 cc. of reagent acetone plus 2.5 cc. of 10 per cent ammonia, shake one hour, filter, take 100 cc. of the filtrate, which represents 10 Gm. of the drug, and evaporate under a current of air to approximately 25 cc. Add 10 cc. of distilled water plus enough tartaric acid to a pH of 3. Shake the acid-acetone-water solution with two 50-cc. portions of ether to remove excess fat and most of the coloring matter, and evaporate off the last traces of ether by vacuum distillation. Make the solution alkaline to a pH of 8 with sodium bicarbonate, allow to stand for 10 minutes, filter, and wash the container and filter paper with two 10-cc. portions of a 1 per cent solution of sodium bicarbonate. The precipitate on the filter paper represents most of the water-insoluble alkaloids (ergotoxine-like), and the filtrate contains the water-soluble alkaloid (ergonovine). Dissolve the precipitate on the filter paper in 10 cc. of a 2 per cent solution of tartaric acid, and assay by the Broom-Clark Method and colorimetrically for the ergotoxine-like alkaloids, with ergotoxine ethanesulfonate as the standard.

"In a separatory funnel, shake the filtrate (ergonovine) with two 50-cc. portions of carbon tetrachloride (10 minutes for each shaking) to remove the last traces of the ergotoxine-like alkaloids, run off the carbon tetrachloride, and remove all traces of carbon tetrachloride from the ergonovine solution by vacuum distillation (a little heat may be necessary). Add sodium chloride to saturation and shake with successive portions of ether until there is no blue color (*p*-dimethyl-aminobenzaldehyde reagent). Evaporate off the ether by air current in 10 cc. of distilled water made slightly acid with tartaric acid and assay biologically (isolated rabbit's uterus) and colorimetrically with pure ergonovine maleate as the standard."

The method described above was first tested on various mixtures of the pure alkaloids, ergotoxine ethanesulfonate and ergonovine maleate dissolved in the ammoniated acetone. As shown in Table I, both biologically and colorimetrically, an average of

more than 90 per cent of either alkaloid was accounted for. Thus, the results show that, working with solutions of the pure alkaloids, a quantitative determination of both alkaloids is possible.

With the crude ergot drug, the method was found to be useful in evaluating the ergonovine content. In the commercial isolation of ergonovine maleate, this method has been most helpful in the selection of good batches and in the yield of ergonovine. As shown in Table II, the thirty-seven lots of ergot contained from 2–20 mg. of ergonovine per 100 Gm. of crude ergot. The colorimetric method on the average gave a higher potency value for ergonovine than the oxytocic assay (isolated rabbit's uterus).

For the ergotoxine-like alkaloids in the drug and unlike that of the pure ergotoxine ethanesulfonate (Table I), the method was not so successful. The various solvents, gasoline or petroleum ether (used to defat the drug), and ether washings (to remove the coloring matter) also extracted some of the ergo-

Table I.—Mixtures of Pure Ergonovine Maleate and Pure Ergotoxine Ethanesulfonate

Experiment No.	Mixture of Pure Alkaloids		Colorimetric Method		Bioassay Methods	
					Broom-Clark	Oxytocic
	Ergotoxine Ethanesulfonate, mg. per 100 Cc.	Ergonovine Maleate, mg. per 100 Cc.	Ergotoxine Ethanesulfonate, mg. per 100 Cc.	Ergonovine Maleate, mg. per 100 Cc.	Ergotoxine Ethanesulfonate, mg. per 100 Cc.	Ergonovine Maleate, mg. per 100 Cc.
1	50.0	10.0	46.0	9.4	46.0	9.0
2	50.0	20.0	49.0	19.4	52.0	20.0
3	50.0	25.0	50.0	24.5	53.0	22.0
4	50.0	15.0	47.5	14.4	49.5	14.6
5	30.0	10.0	24.1	9.0	29.0	8.5
6	48.0	10.0	48.0	9.4	54.0	9.5
7	50.0	5.6	40.0	5.0	47.0	4.4
8	50.0	7.0	50.0	6.0	42.0	6.8
9	50.0	13.0	45.0	12.0	43.0	9.7
10	50.0	10.0	50.0	10.0	43.0	9.0

Table II.—Crude Ergot

Lot No.	Colorimetric Method						Biological Methods					
	Ergotoxine Ethanesulfonate Equivalent, mg. per 100 Gm.					Ergonovine Maleate Equivalent, mg. per 100 Gm.	Boom-Clark Method Ergotoxine Ethanesulfonate Equivalent, mg. per 100 Gm.					Ergonovine Maleate Equivalent, mg. per 100 Gm.
	Defatted (Gasoline)	Ether Washings	Total Alkaloids	After Pptn. with $NaHCO_3$	CCl_4 Washings		Defatted (Gasoline)	Ether Washings	Before Pptn.	After Pptn. with $NaHCO_3$	CCl_4 Washings	
1	...	...	...	...	9.2	9.0	3.00	6.50	60.0	45.0	4.00	8.0
2	...	...	...	...	12.0	9.0	4.75	6.15	65.0	50.0	4.00	10.0
3	...	...	...	...	9.6	11.0	3.35	9.50	70.0	50.0	2.90	13.0
4	...	...	...	...	8.0	18.4	2.50	12.00	65.0	47.5	1.35	15.0
5	...	...	...	...	10.0	15.0	2.30	13.50	71.0	56.5	6.70	10.0
6	...	...	...	...	13.0	13.0	7.40	12.35	75.0	50.0	5.00	10.0
7	...	...	...	140.0	...	15.0	...	...	...	113.0	...	12.5
8	...	...	...	...	...	18.5	...	...	...	96.0	...	16.0
9	...	...	...	131.0	...	15.0	...	...	...	146.0	...	14.0
10	...	...	...	180.0	...	12.5	...	...	...	130.0	...	10.0
11	...	...	...	131.0	...	22.5	...	...	...	140.0	...	20.0
12	...	...	...	180.0	...	21.5	...	...	...	142.0	...	20.0
13	...	...	...	...	...	25.0	...	...	...	162.0	...	20.0
14	...	...	...	...	...	20.0	...	...	...	137.5	...	19.0
15	...	...	...	...	...	22.5	...	...	...	137.5	...	20.0
16	...	...	...	225.0	...	24.5	...	...	...	170.0	...	20.0
17	...	...	...	...	...	23.5	...	...	...	...	...	20.0
18	...	...	...	...	...	25.5	...	...	...	...	...	20.0
19	...	...	...	...	...	23.5	...	...	...	...	...	20.0
20	...	...	...	...	...	20.0	...	...	...	95.0	...	20.0
21	...	...	...	100.0	...	23.0	...	...	...	85.0	...	20.0
22	...	...	...	...	...	10.0	...	...	...	108.0	...	8.0
23	...	...	...	...	...	22.5	...	...	...	80.0	...	20.0
24	...	...	...	...	...	19.0	...	...	...	55.0	...	16.0
25	...	...	...	...	...	12.3	...	...	...	...	...	11.9
26	...	...	...	...	...	7.0	...	...	...	...	...	5.8
27	...	...	...	...	...	13.5	...	...	...	...	...	12.5
28	...	...	...	...	...	13.5	...	...	...	...	...	12.5
29	...	...	...	...	...	11.0	...	...	...	...	...	6.7
30	...	...	...	...	...	3.0	...	...	...	...	...	2.0
31	...	...	...	...	...	3.0	...	...	...	...	...	2.0
32	...	...	...	...	...	3.0	...	...	...	...	...	2.0
33	...	...	...	...	...	12.1	...	...	...	...	...	12.5
34	...	...	...	...	...	10.9	...	...	...	...	...	10.0
35	...	...	...	...	...	11.6	...	...	...	...	...	12.5
36	...	...	...	...	...	14.5	...	...	...	...	...	13.3
37	...	...	...	...	...	21.5	...	...	...	...	...	20.0

toxine-like alkaloids, but only slight traces, if any, of the ergonovine. Incidentally, the more fats and waxes the drug contains, the more difficult it is to separate the alkaloids. The filtrate fraction (ergonovine) after precipitation with sodium bicarbonate still has enough of the ergotoxine-like alkaloids to prevent an oxytocic test. These alkaloids were removed by shaking with carbon tetrachloride (Table II, columns 6 and 12). As previously reported by Hampshire and Page (12), the ergonovine base is insoluble and the ergotoxine base is soluble in carbon tetrachloride. Thus, in the crude drug, ergonovine is more accurately evaluated by this method than the ergotoxine-like alkaloids.

Table III.—Examination of Thirty-six Commercial Fluidextracts of Ergot U. S. P.

	Colorimetric Method					Biological Methods					
						Broom-Clark Method					
	Ergotoxine Ethanesulfonate Equivalent, mg. per 100 cc.				Ergonovine Maleate Equivalent, mg. per 100 Cc.	Ergotoxine Ethanesulfonate Equivalent, mg. per 100 Cc.					Ergonovine Maleate Equivalent, Mgm. per 100 Cc.
F. E. No.	Ether Washings	Total Alkaloids	After Pptn. with $NaHCO_3$	CCl_4 Washings		Original F. E Before	Ether Washings	After Ether Washings	After Pptn. with $NaHCO_3$	CCl_4 Washings	
1	None	111.76	50.88	30.10	11.00	50.0	None	50.0	44.0	5.00	8.0
2	None	95.92	51.80	32.30	10.40	50.0	None	48.5	47.0	3.50	6.0
3	Trace	122.96	52.20	30.00	9.50	50.0	None	55.0	40.0	7.00	4.0
4	Trace	208.60	62.40	36.40	13.70	70.0	None	65.5	50.0	15.00	8.0
5	None	81.60	20.00	16.80	7.70	24.0	None	23.0	20.0	4.25	3.0
6	None	107.20	35.00	25.70	11.00	41.0	None	40.0	30.0	5.00	6.0
7	None	160.00	46.00	36.00	22.80	50.0	None	50.0	40.0	6.35	8.0
8	None	77.60	20.60	23.00	12.20	12.0	None	13.0	11.0	2.55	4.0
9	None	177.12	64.00	41.60	17.20	44.0	None	45.0	40.0	5.65	6.5
10	None	116.52	26.40	19.40	8.00	37.5	None	38.0	35.0	2.95	2.5
11	None	101.50	58.20	29.30	10.00	55.0	None	54.0	50.0	4.00	8.5
12	None	93.90	43.20	17.50	8.60	45.0	None	43.0	38.0	7.00	5.0
13	...	...	49.50	...	6.52	...	...	...	30.0	...	5.0
14	...	...	45.00	...	7.15	...	...	...	27.5	...	5.7
15	...	...	40.00	...	5.10	...	...	...	26.0	...	5.0
16	...	...	51.50	...	5.20	...	...	...	31.0	...	5.0
17	...	...	42.50	...	5.56	...	...	...	26.5	...	3.2
18	...	...	45.50	...	6.00	...	...	...	38.5	...	5.2
19	...	...	51.50	...	5.94	...	...	...	30.5	...	3.0
20	...	...	55.50	...	8.16	...	...	...	30.0	...	5.1
21	...	...	52.50	...	12.10	...	...	...	36.5	...	10.0
22	...	...	60.50	...	13.60	...	...	...	37.5	...	11.0
23	...	...	58.50	...	5.55	...	...	...	41.5	...	5.0
24	...	...	62.20	...	7.95	...	...	...	37.8	...	2.5
25	...	...	62.20	...	6.85	...	...	...	40.0	...	5.0
26	...	...	57.20	...	13.70	...	...	...	37.0	...	11.0
27	...	...	89.50	...	11.60	...	...	...	43.5	...	10.0
28	...	...	89.50	...	18.90	...	...	...	44.5	...	18.2
29	...	...	61.50	...	14.00	...	...	...	42.5	...	8.0
30	...	...	70.00	...	13.00	...	...	...	37.0	...	8.0
31	...	...	80.74	...	17.50	...	...	...	45.0	...	10.0
32	...	...	56.08	...	16.00	...	...	...	40.0	...	8.0
33	...	...	61.44	...	18.00	...	...	...	42.5	...	10.0
34	...	...	36.24	...	13.00	...	...	...	30.0	...	8.0
35	...	...	19.90	...	13.00	...	...	...	15.0	...	9.0
36	...	...	60.48	...	13.00	...	...	...	40.0	...	9.0

Method for the Fluidextract of Ergot, U. S. P.—In the preparation of the fluidextract, 40 per cent alcohol is used. This highly colored dilute alcoholic solution required a slightly different procedure. The method may read as follows:

"Place 12.5 cc. of the fluidextract of ergot U. S. P. in a flask suitable for use in a mechanical shaker. Add 5 cc. of 10 per cent ammonia plus 125 cc. of reagent acetone, shake for one hour, filter, and take 100 cc. of the filtrate, which represents 10 cc. of the fluidextract, and evaporate under a current of air. to dryness (it is necessary to remove all the alcohol). Add 25 cc. of distilled water, make alkaline to a pH of 8, plus 7.5 Gm. of sodium chloride, and shake with three separate 50-cc. portions of ether (10 minutes for each shaking). Under a current of air evaporate the combined ether shakings into approximately 25 cc. of a 2 per cent solution of tartaric acid. To remove coloring matter, shake the acid solution with two 50-cc. portions of ether. Evaporate off the traces of ether by vacuum. Make the acid solution alkaline with sodium bicarbonate to a pH of 8, allow to stand for 10–15 minutes, filter, and wash the container and filter paper with two 10-cc. portions of a 1 per cent solution of sodium bicarbonate. The precipitate on the filter paper represents most of the water-insoluble alkaloids (ergotoxine-like) while the filtrate contains the water-soluble alkaloid (ergonovine). Dissolve the precipitate on the filter paper in 10 cc. of a 2 per cent solution of tartaric acid and assay by the Broom-Clark Method. To remove all traces of the ergotoxine-like alkaloids, shake the filtrate (ergonovine) with two portions of 50 cc. of carbon tetrachloride. After separation, evaporate the last traces of carbon tetrachloride by vacuum (a little heat may be necessary). Saturate

the ergonovine solution with sodium chloride and shake with successive portions (50 cc.) of ether until free from blue color (*p*-dimethyl-aminobenzaldehyde reagent). Finally, evaporate the combined ether fractions into 10 cc. of distilled water, remove last traces of ether by vacuum, pH to 3 with tartaric acid, wash with 50 cc. of ether (to remove last traces of color), again remove ether fumes by vacuum, and assay for ergonovine, biologically and colorimetrically, with pure ergonovine maleate as the standard."

As shown in Table III, in the fluidextract the ergonovine content varied from 2.5–18.2 mgm. per 100 cc. (isolated rabbit's uterus) and 5.1–18.9 mgm. per 100 cc. colorimetrically. On the average, the color test gave a higher value than the bioassay method. Thus, this method is useful in determining the ergonovine potency (both biologically and colorimetrically) in the fluidextract.

For the ergotoxine-like alkaloids in the fluid extract, the method appeared to be more quantitative than for the same alkaloids in the crude drug. Physiologically, the results in Table III (column 7 [original assay] and column 9 [after ether washing to remove color]) checked reasonably close, showing that the ether did not extract (unlike that found in the crude drug) any of the water-insoluble alkaloids. This difference in the drug and fluidextract is probably due to the fact that the fluidextract is more thoroughly defatted than the crude drug. Colorimetrically, the results were not in agreement with the bioassay values. In the fluidextract, therefore, it is possible to evaluate the ergotoxine-like alkaloids biologically but not colorimetrically.

In view of the wide variation in the ergonovine content (2.5–18.2 mgm. per 100 cc.) and the ergotoxine-like alkaloids (12–70 mgm. per 100 cc.) of fluid extracts of ergot U. S. P., and the pharmacological evidence (11) of antagonism between these alkaloids, the U. S. P. Cock's Comb Method is obviously of little value for potency determination. It is also well known among bioassayists that the fluidextract is not a stable preparation. Furthermore, ergonovine now plays an important role in the practice of obstetrics and, together with ergotoxine or ergotamine, is considered the chief alkaloid in ergot. These useful alkaloids are now available in pure form. Thus, the uncertainty of potency and of stability of the fluidextract, as well as the availability of its important alkaloids in pure form, leads one to believe that the fluidextract of ergot U. S. P. has lost much of its usefulness as a true representative of ergot preparations.

SUMMARY

1. An assay process for crude ergot and fluidextract of ergot U. S. P. is described, in which ergonovine is completely separated from the ergotoxine-like alkaloids.

2. By this method thirty-seven lots of crude ergot drug and thirty-six various commercial fluidextracts of ergot U. S. P. were studied.

3. The ergonovine content in these preparations was more accurately evaluated by this method than the ergotoxine-like group.

4. For potency tests on the average, the colorimetric method gave higher values than the biological methods. The isolated rabbit's uterus was used for ergonovine, and the Broom-Clark Method for the ergotoxine-like alkaloids.

5. In the commercial isolation of ergonovine maleate, this method has been helpful in the selection of the crude ergot and in the yield expected during the process of manufacture.

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"Brevity in writing is the best insurance for its perusal."—Virchow