FLUIDEXTRACT OF ERGOT: EFFECT OF ACIDITY ON BIOLOGIC ACTIVITY AS DETERMINED BY U. S. P. 1935 REVISED ASSAY.*

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INTRODUCTION.

Fluidextract of Ergot has been a subject of extensive investigation over a period of many years. Revision Committees of the United States Pharmacopœia have agreed through several revisions upon the advisability of using an acid medium in the extraction of ergot.

In recent years with more effectively controlled methods of physiological assay available, attention has been directed not only to the influence of acid upon the yield of ergot alkaloids but also to its influence upon the stability of these alkaloids. The results of this work are reflected in the use of hydrochloric acid in both the extraction and dilution menstrua in the recent interim revision monograph on Fluidextract of Ergot of the U. S. P. X.

Early in 1930 a series of experiments was undertaken with the object of further determining the possible value of varying the amount of acid used in the extracting medium. A series of fluid extracts was prepared from the same lot of defatted drug. The menstrua contained, respectively, 10, 20, 30, 40 and 50 cc. of hydrochloric acid in 1000 cc. dilute alcohol.

For purpose of minimizing influences other than acidity the extracts were stored in the dark in small, well-filled, sealed containers. Samples were assayed over a period of approximately three years.

The general indication of the results was to the effect that the greater the acidity of the menstruum and therefore of the resultant extract the greater was the initial and maintained activity of the extract; this apparently confirmed previously expressed opinions and observations as to the influence of acidity on the stability of Fluidextract of Ergot. However, certain unexplained irregularities in the findings and a careful scrutiny of the numerous cocks comb assays of samples which were carried out during this period suggested the possibility that the increased acidity of some of the extracts might in itself be a factor in the observed higher activity of the samples as determined by the cocks comb assay.

With this in mind experiments were carried out for the purpose of determining whether or not the acidity of Fluidextract of Ergot is a factor in the Bio-assay value.

EXPERIMENTAL.

Assay Method—Procedure and Method of Calculation.—The tests were carried out on flocks of 35–45 white leghorn cockerels weighing approximately 2 kilos each, an entire flock being used for one day's test. The flock was divided into four equal groups, two of which received doses of the standard sample and two the test samples. The birds in each group all received the same dose, two dose levels being employed for both standard and test sample. The doses, which ranged from 0.1 to

* Scientific Section, A. PH. A., Portland meeting, 1935.

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0.2 cc. per kilo of bodyweight, were injected into the breast muscle and the effect, expressed as the percentage of the comb area in which darkening occurred, was recorded one, one and one-half and two hours afterward. For purposes of calculation the average of the two highest of these readings was used. Using the group averages, the activity of the test sample relative to the standard was obtained from a dose-effect graph in which the logarithms of the doses were plotted against the effects expressed as percentage of the comb area in which darkening occurred. After an interval of ten days or more the test sample and the standard were again compared, using the same flock of birds but using for the test sample the birds employed for the standard in the first day's test and for the standard the birds previously used for the test sample.

PREPARATION OF SAMPLES.

In four series of experiments sets of samples of Fluidextract of Ergot and ergotoxine ethanesulphonate were prepared by additions of hydrochloric acid, tartaric acid or sodium hydroxide, as required, to obtain specific adjustments of p_H.

For Experiment I.—To one volume Fluidextract Ergot B, 188 (made with menstruum containing 40 cc. hydrochloric acid per liter) (p_H 3.0) was added:

(*a*) 0.225 volumes of 1 *normal* NaOH to neutralize one-half of the hydrochloric acid used in the extraction—p_H of adjusted fluid extract was 5.0.

(*b*) 0.45 volumes of 1 *normal* NaOH to neutralize all of the hydrochloric acid used in the extraction—p_H of adjusted fluid extract 6.8.

For Experiment II.—(*a*) A Fluidextract Ergot (1 cc. of extract equivalent to 1 Gm. of drug) was prepared by extraction of the ground drug with 45% aqueous solution ethyl alcohol; p_H of the extract was 5.7.

(*b*) A portion of the 45% alcoholic extract was adjusted to p_H 3.2 by addition of concentrated hydrochloric acid.

For Experiment III.—(*a*) A Fluidextract Ergot was prepared with 45% solution of ethyl alcohol in exactly the same manner as Experiment II. The p_H of the extract was 5.7.

(*b*) Concentrated hydrochloric acid was diluted with 40% alcohol to 0.16*N*.

For Experiment IV.—(*a*) A solution of ergotoxine ethanesulphonate was prepared by dissolving 0.5 mg. per cc. of the salt in 1% aqueous tartaric acid; p_H of solution 2.0.

(*b*) A solution of ergotoxine ethanesulphonate was prepared by dissolving 0.5 mg. per cc. of the salt in 0.25% aqueous tartaric acid, p_H of solution 3.1.

(*c*) An aqueous solution of ergotoxine ethanesulphonate was prepared by suspending 0.5 mg. per cc. of the salt in double distilled water and adding a trace of tartaric acid to clarify the solution; p_H of solution 5.0.

RESULTS.

The physiological activities of the above-described experiments were determined by the assay method given and the results calculated in terms of activity ratios as follows:

Experiment I.—Fluidextract of Ergot compared to same with acid in extract one-half neutralized, and acid in extract completely neutralized. Alkali added immediately before injections.

Sample.	p_H of Sample.	Dose Injected Cc./Kg.	% Average Area Cyanosis for Number Birds Indicated in Parentheses. First Test.	Second Test.	Third Test.	Relative Activity Calculated from Lot-Dose—% Area Effect Correlation Graphs. First Test.	Second Test.	Third Test.	Weight Av. Value Relative Activity.
Fldext. Ergot B No. 188	3.0	0.13	33.3 (10)	30.2 (10)	19.2 (9)	1.00	1.00	1.00	1.00

Same. 0.225 Vol.									
N—NaOH	5.0	0.13	20.3 (10)	25.5 (9)	19.0 (10)	0.677	0.869	1.00	0.85
Fldext. Ergot B		0.9	42.5 (11)			1.00			
No. 188	3.0	0.13	33.3 (10)	30.2 (10)	19.2 (9)	1.00	1.00	1.00	1.00
		0.11	43.2 (10)	34.5 (10)		1.00	1.00		
Same. 0.45 Vol.		0.9	29.6 (7)			0.542			
N—NaOH	6.8	0.13	15.3 (9)	21.2 (10)	19.5 (10)	0.577	0.762	1.00	0.683
		0.11	19.5 (10)	20.3 (8)		0.500	0.662		(6 tests)

Experiment II.—Fluidextract of Ergot—prepared with 45% neutral alcohol compared to same with p_H adjusted to 3.2.

Sample.	p_H of Sample.	Dose Injected.	% Av. Area Cyanosis for Number Birds Indicated in Parentheses. First Test.	Second or "Crossover" Test.	Relative Activity Calculated from Lot—% Area Effect Correlation Graphs. First Test.	Second Test.	Weight Av. Value Relative Activity.
45% alcohol extract ergot, no adjustment	5.7	0.25 cc./Kg.	15.7 (10)	27.3 (11)	1.00	1.00	1.00
		0.30	15.5 (11)	38.2 (11)	1.00	1.00	
45% alcohol extract ergot, p_H adjusted by addition concd. HCl	3.2	0.25	26.6 (11)	49.2 (9)	1.32	1.44	1.32
		0.30	38.2 (11)	46.8 (11)	1.33	1.117	

Experiment III.—Fluidextract of Ergot prepared with 45% neutral alcohol compared to same with 0.16*N* HCl in 40% alcohol injected in opposite breast muscle of test birds.

Sample.	p_H of Sample.	Dose Injected.	% Av. Area Cyanosis for Number Birds Indicated in Parentheses. First Test.	Second or "Crossover" Test.	Relative Activity Calculated from Lot—% Area Effect Correlation Graphs. First Test.	Second Test.	Weight Av. Value Relative Activity.
45% alcohol extract ergot	5.7	0.3	28.0 (11)		1.00		1.00
		0.45	30.3 (9)		1.00		
No adjustment " " injected in left breast muscle	5.7	0.3	25.8 (9)		1.073		0.93
Dil. HCl—0.16*N* in 40% alcohol injected in right breast muscle of same birds		0.45	38.2 (7)		0.778		

Experiment IV.—Solution ergotoxine ethanesulphonate 0.5% in double-distilled water—trace of tartaric acid to clarify compared to 0.5% ergotoxine ethanesulphonate in 1.0% tartaric acid.

Ergotoxine ethanesulphonate 0.5% in double-distilled water (trace tartaric acid added to clarify)	5.0	0.08	21.7 (13)	25.0 (13)	1.00	1.00	1.00
		0.12	30.5 (9)	49.7 (12)	1.00	1.00	
Same in 1.0% tartaric acid	2.0	0.08	24.4 (13)	37.6 (13)	1.16	1.23	1.28
		0.12	47.6 (9)	63.2 (9)	1.48	1.25	

Experiment V.—Same as IV in 1% aqueous tartaric acid compared to 0.5% E. E. S. in 0.25% tartaric.

3.1	0.075	23.8 (12)	1.00	1.00
	0.10	33.4 (11)	1.00	
2.0	0.075	21.9 (11)	0.946	
	0.10	31.1 (11)	0.930	0.938

SUMMARY AND CONCLUSIONS.

1. Neutralization in two stages (to p_H 5.0 and to p_H 6.8) of an acid Fluidextract of Ergot (p_H 3.0) at the time of injection resulted in a decrease proportional to the amount of alkali added, of the apparent activity of the sample as determined by the U. S. P. X 1935, Interim Revision Cocks Comb Physiological Test.

2. Since adjustment of the p_H of a clear neutral alcohol extract of ergot from 5.7 to 3.2 by addition of hydrochloric acid resulted in an increase of 32 per cent in the apparent activity of the sample, it is concluded that the apparent decrease in activity which followed neutralization of the acid fluid extract was not related to any effect of the added alkali upon the sample.

3. Since simultaneous injection of an amount of hydrochloric acid—equivalent to that present in U. S. P. X Fluidextract of Ergot 1935, Interim Revision into the breast muscles of a series of cockerels opposite to that in which a neutral alcohol Fluidextract of Ergot was injected did not increase the apparent activity of the fluid extract as compared to the activity observed in a second series of cockerels simultaneously injected with the neutral Fluidextract of Ergot alone—it is concluded that the observed influence of the degree of acidity of Fluidextract of Ergot upon the apparently physiological activity of the same is not related to any systemic factor but rather is to be explained in terms of rate of absorption of the ergot alkaloids from the site of injection in the breast muscle.

4. Ergotoxine ethanesulphonate solution prepared according to U. S. P. X 1935, Interim Revision to contain 0.5 mg. per cc. dissolved in aqueous solution of tartaric acid 1 in 100 (p_H 2.0) showed an apparent physiological activity 28 per cent greater than that of an aqueous solution of ergotoxine ethanesulphonate 0.5 mg. per cc. p_H 5.0 to which a trace of tartaric acid had been added to clarify the solution. Comparison of the 0.5 mg. per cc. solution of ergotoxine ethanesulphonate solution in 1 per cent tartaric acid to ergotoxine ethanesulphonate solution 0.5 mg. per cc. dissolved in aqueous tartaric acid 0.25 in 100 (p_H 3.1) showed the latter to have an apparent activity 6 per cent more than the former. It is concluded that the apparent activity of ergotoxine ethanesulphonate, as determined by the U. S. P. X 1935, Interim Revision Cocks Comb Test, is influenced in the same way by the acidity of the solution in the same manner as is Fluidextract of Ergot but probably to a lesser extent.

5. It is concluded from the results presented in this communication that the U. S. P. X Bio-assay value of samples of Fluidextract of Ergot may be materially influenced by the acidity of the solutions which are injected in this assay. This suggests the desirability of including in the U. S. P. XI monograph on Ergot a specification in the assay procedure as to adjustment of ergot solutions for purpose of injection to a stated p_H.

The biological assays and tabulations used herein were prepared by the Biological Research Laboratories of E. R. Squibb & Sons, and I gratefully acknowledge their assistance.