THE STANDARDIZATION AND STABILIZATION OF ERGOT PREPARATIONS.*

THE STUDY OF BIOLOGICAL METHODS OF ASSAYING ERGOT PREPARATIONS AND THE HYDROGEN-ION CONCENTRATION FACTOR.

A Preliminary Report—Paper VII.

BY EDWARD E. SWANSON.[1]

1. INTRODUCTION.

The principle constituents of ergot are considered to be ergotoxine, ergotamine, tyramine, acetylcholine and histamine. Ergotoxine and ergotamine, however, are the only constituents that will produce the characteristic cyanotic and gangrenous comb of the cock.

Numerous methods of assaying ergot have been studied and reported, but only two or three methods show agreement as to relative values. Some of the methods reported are:

(*a*) the Isolated Guinea-Pig Uterus Method,
(*b*) the Blood Pressure Method,
(*c*) the Vasomotor Reversal Method (Barger & Dale) (1),
(*d*) the Cat's Uterus *in situ* Method,
(*e*) the Cock's Comb Method U. S. P. (Edmunds & Hale) (2),
(*f*) the Isolated Rabbit's Uterus Method (Broom & Clark) (3).

Edmunds and Hale (2) found that the Cock's Comb Method and Guinea-Pig Uterus Method are in close agreement in measuring the activity of ergot, the blood pressure method and the cat's uterus *in situ* method gave great individual variation in dogs and cats. Wood and Hofer (4) reported considerable variation in cats by the vasomotor reversal method. Broom and Clark (3) found considerable variation in some of the methods and concluded that the isolated guinea-pig uterus and the cat's uterus *in situ* measured the amine content of ergot, whereas, the cock's comb, vasomotor reversal action, and the isolated rabbit's uterus all measured the alkaloidal content of ergot, namely, ergotoxine and ergotamine. Broom and Clark further concluded that even though the rabbit's uterus and cock's comb are correlative in determining the alkaloidal content of ergot, the Cock's Comb Method shows greater variation than the Rabbit's Uterus Method, and suggested the later as the logical method for assaying ergot preparations.

More recently there have been a number of methods reported based on the epinephrine reversal action on the rabbit's uterus, Broom and Clark (3). Planelles

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[1] Department of Pharmacology, The Lilly Research Laboratories, Indianapolis, Indiana.

(5) used guinea-pig intestines; Masuda (6) tried the frog vessels and Raymond-Hamet (7) applied the renal vasomotors. Still more recently Nelson and Pattee (8) and Pattee and Nelson (9) have reported on the Isolated Rabbit's Uterus and also compared this method with the U. S. P. Cock's Comb Method. These authors found that the two methods yield results practically identical, that is, both methods determine the alkaloidal content of ergot. They also found that the U. S. P. Standard Fluidextract of Ergot by the Broom and Clark Method is approximately the equivalent of a 0.05 per cent solution of the specific alkaloids, which is about one-half the value of the standard set up by the Research Laboratory of the British Pharmaceutical Association as suitable for fluidextracts.

Considering the above review of the literature, it seems important to study the value of the Epinephrine Reversal Method (Broom and Clark) in the commercial assaying of ergot preparations and to compare this method with the Cock's Comb Method U. S. P.

Nelson and Pattee (8) and Pattee and Nelson (9) reported that there is considerable variation in potency of ergot preparations, particularly, the solution of ergot for hypodermic use.

This article is the result of a comparative study of the Cock's Comb Method U. S. P. and the Broom and Clark Method, particularly of the latter, on a number of Fluidextracts of Ergot U. S. P. samples and solutions of ergot for hypodermic use, and also the hydrogen-ion concentration factor in relation to stability and deterioration.

2. METHODS OF ASSAY.

The Cock's Comb Method, U. S. P. is a well-known method and need not be described here. Gittinger and Munch (10) reported on this method and their results are in close agreement with our own findings.

The Rabbit's Uterus Method (Broom and Clark) involves the use of strings of uterine muscle in a constant temperature bath; the strips are immersed in Locke-Ringer's solution oxygenated by air and the muscles or strips arranged so as to record their contractions. Epinephrine reactions are first determined until these contractions are consistent to a certain degree, and then the ergot to be assayed is given to one muscle and the standard ergot to the other muscle. The ergot is allowed to remain in the cylinders five minutes, and then washed. The washing is important because Pattee and Nelson (9) has shown the antagonistic action of histamine. The amines, tyramine and acetylcholine, are now being studied and will be reported in another article. Following the washing or flushing of the muscle with fresh salt solution the same amount of epinephrine is injected or increased amounts of epinephrine are injected until the contractions are equal to the contractions of epinephrine before ergot paralysis. These factors will be described later.

3. SELECTION OF A MUSCLE.

The writer confirms the results of Burn (11) and Pattee and Nelson (9) in the use of large rabbits. The segregation of rabbits is helpful in obtaining suitable uteri. This also eliminates the influence of multiparous, post-partum and parturition factors. The segregation of young rabbits either virgin or monoparous, allowed to reach the weight of 3 or 4 Kg., is helpful in the choice of a suitable uterus.

The size of the individual uterus, regardless of the weight of the rabbit, is variable, but this is not so great when isolation is employed. Under these conditions of selection the uterine contractions are slow with little normal rhythm, resembling the guinea-pig uterus in the standardization of pituitary extract. A uterus with strong normal rhythmic contractions is usually too rapid in its contractions when stimulated with epinephrine and is not, in our findings, considered so reliable. Our experience favors the use of the whole uterus, that is, each fallopian tube is divided into two equal parts—thus supplying four strips. If the uterus is large, longitudinal cuts are made and divided into eight or more strips. Our findings favor the same degree of selection of a rabbit uterus as in the selection of guinea-pig uterus for pituitary testing. The writer prefers strips so cut as to give contractions of 40 mm. to 60 mm.

Much valuable time can be saved by external examination of the vaginal orifice as stated by Pattee and Nelson (9).

The selection of a choice uterus requires experience. It is the most difficult part of this method. For this reason the commercial application of this method in standardizing ergot preparations is still questionable.

4. TECHNIQUE OF ASSAYING.

Having selected a suitable uterus, the following three methods of technique have been studied:

A. *Complete paralysis* of the uterus by ergot.

B. *Partial paralysis* of uterus.

C. *The ratio of epinephrine stimulation to ergot paralysis.*

Method A.—The application of large doses of ergot to cause complete paralysis or inhibition to epinephrine stimulation is not consistent for all uterine muscle, that is, some uteri require more or less ergot to produce inhibition. It is possible, however, by this technique to assay an ergot preparation by determining on a number of muscles the minimum amount of ergot in comparison to a standard ergot as a control that will cause inhibition or paralysis. (See Fig. 1, the lower tracing.)

Method B.—Partial paralysis involves the use of small doses of ergot (both standard and unknown) that will only partially inhibit the epinephrine stimulation. Gradually decreasing doses of epinephrine are injected, until quantitative contractions are obtained and then a small dose of ergot is injected, five minutes duration allowed, washed once or twice and finally the same amount of epinephrine is injected as before ergot application. The amount of inhibition is measured, compared with the control and the amount of reduction in contraction calculated. Injections of epinephrine are repeated until the contractions of the uterus return to constant height with the same amount of epinephrine. When the contractions are constant ergot is again injected and the same technique repeated as above. Thus small doses of ergot or partial paralysis permits the injection of several doses of ergot on the same uterus, and by alternating the unknown and standard on the same muscle one can obtain fairly accurate comparisons, see Figs. 1, 2, 3 and 4.

Method C.—This technique determines the amount of paralysis in terms of epinephrine, that is, following the ergot paralysis the amount of epinephrine in-

jected is increased until the contraction equals in height the contraction of epinephrine before ergot was injected. Thus the amount of increase in epinephrine depends upon the amount of ergot injected or the degree of paralysis caused by ergot. As in Figs. 2 and 3, 1 cc. (1–100) Fluidextract of Ergot U. S. P. produced a 1 to 2 ratio in epinephrine and 0.005 mg. of ergotoxine produced a 1 to $2^2/_3$ ratio. Therefore this Fluidextract of Ergot U. S. P. contained 0.0375% of alkaloid in terms of ergotoxine phosphate.

All of the above three methods give fairly accurate results. Methods B and C are more reliable in that the same muscle may be used for the standard and the unknown. Method C, theoretically, seems to be the most reliable because ergot paralysis is balanced against epinephrine stimulation. The assays in the following tables are based on Methods A and B. Method C is being more extensively studied and will be reported in a later article.

5. EXPERIMENTAL DATA.

The following data represents a number of Fluidextracts of Ergot U. S. P. These samples are manufactured lots, each being assayed by the Cock's Comb and Reversal Uteri (Broom and Clark) Methods.

TABLE I.

Data.	F. E. Ergot, U. S. P., sample no.	Cock's Comb Method, per cent activity.	Reversal Uteri, Broom and Clark. Alkaloidal content.	Per cent activity.
6– 2–27	1	100%	0.050%	100%
8–31–27	2	100%	0.050%	100%
11– 1–27	3	90%	0.045%	90%
10–23–28	4	100%	0.050%	100%
11–10–28	5	80%	0.045%	90%
7–30–28	6	100%	0.055%	110%

These results show that the two methods correlate in determining the alkaloidal content of fluidextracts.

One-half gallon of fluidextract of ergot was reserved from a manufactured lot and divided into eight equal parts. One of the samples or parts contained no acid, to the remaining seven parts various amounts of HCl 36% were added so as to have various hydrogen-ion concentrations. The original assays by the Cock's Comb and Reversal Uteri (Broom and Clark) Methods were 90% and 100%, respectively.

The following table shows the assays of these samples after two years' aging.

TABLE II.

F. E. Ergot, sample no.	P_H.	Cock's Comb Method, per cent activity.	Broom and Clark Method. Alkaloidal content.	Per cent activity.
1	5.35	less 10%	0.001%	2%
2	4.95	16%	0.002%	4%
3	4.51	40%	0.02%	40%
4	3.77	60%	0.03%	60%
5	3.21	80%	0.04%	80%
6	2.69	80% to 90%	0.05%	100%
7	2.26	..	0.05%	100%
8	1.90	90%	0.05%	100%

Inspection of the above table shows that by both methods the results are correlative. The two methods determine the alkaloidal content of ergot. The cock's comb assay is less reliable as the deterioration increases. This may be due to the large amount of ergot that is required to be injected into the breast muscle of the cock.

The hydrogen-ion concentration factor shows that the Fluidextract of Ergot requires a certain amount of acid to prevent deterioration. Sample No. 5 contained the same amount of acid that is required by the U. S. P. A p_H value of 3.00 is necessary to prevent deterioration. See Chart I.

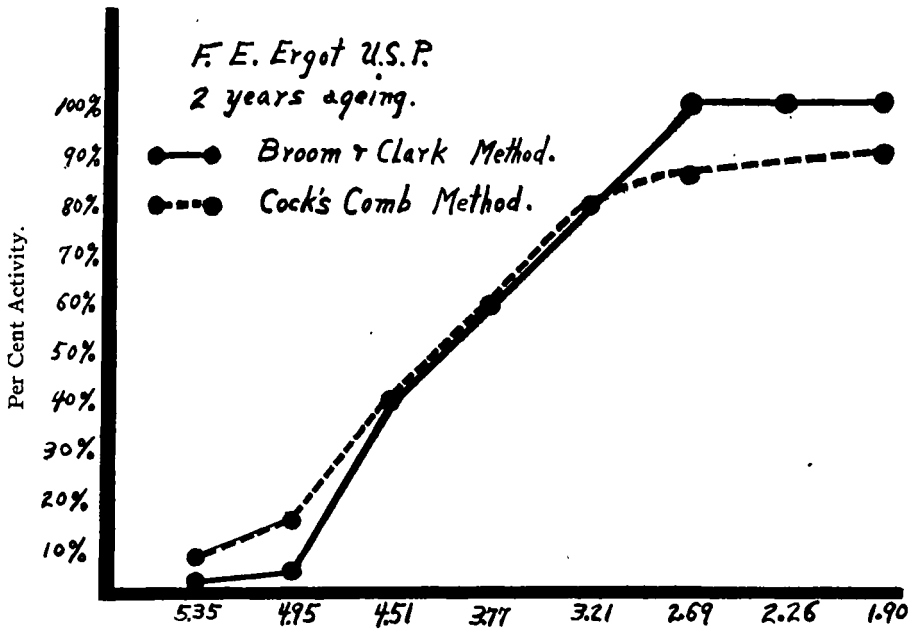

Chart I.—Hydrogen-ion concentration.

A number of solutions of ergot for hypodermic use were assayed by the two methods. The results are as follows:

TABLE III.

Solution no.	Date made.	Date assayed.	Cock's Comb Method.	Broom and Clark. Alkaloidal content.	Broom and Clark. Per cent activity.
1	9-29-26	2-27-28	50% to 60%	0.010%	20%
2	1-14-27	2-27-28	125%	0.040%	80%
3	10-29-27	2-27-28	125%	0.080%	160%
4	11- 2-27	3- 6-28	140%	0.080%	160%

These solutions were made so that 1 cc. contained the equivalent of 2 Gm. of ergot drug. The original assay of the four samples was 200% by the Cock's Comb Method. No assay was made by the Broom and Clark Method. There is no question from inspection of the above table, that solutions of ergot for hypodermic use deteriorate rapidly. This is in agreement with that reported by Pattee and Nelson (9). This deterioration may be a hydrogen-ion concentration factor.

In order to determine the question of the hydrogen-ion concentration a solution of ergot for hypodermic use was prepared August 1928, 1 cc. contained the equivalent of 2 Gm. of drug and assayed 150% by the Cock's Comb method. This solution was divided into six parts; each sample or part was treated with 36% HCl so as to have various hydrogen-ion concentrations. These samples are now being assayed by the two methods to determine stability following a year's aging.

TABLE IV.

Sample no.	p_H.	Date.	Cock's Comb Method, %.	Broom and Clark Method. Alkaloidal content.	Per cent activity.
1	4.30	8–20–28	150%	0.08%	160%
2	3.20	8–20–28	...	....	...
3	2.90	8–20–28	...	....	...
4	2.40	8–20–28	...	....	...
5	1.90	8–20–28	...	....	...
6	1.75	8–20–28	150%	0.08%	160%

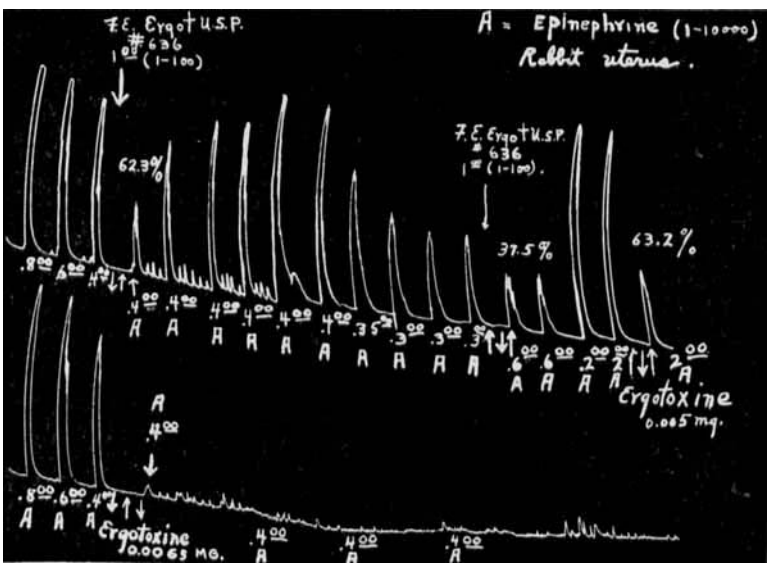

Fig. 1.—The upper tracing represents Methods B and C. The lower tracing represents Method A.

The results of the above assays following one year's aging will be reported later.

6. ERGOTOXINE AND ERGOTAMINE RELATIONSHIP.

In regard to the alkaloids of ergot, ergotoxine and ergotamine, the writer is in agreement with Pattee and Nelson that ergotoxine is distinctly more active than ergotamine. The average figures of more than a hundred tests show the following equivalents: 1 mg. Ergotoxine base is the equivalent of 1.3 mg. Ergotamine base. In Figs. 1, 2, 3 and 4 the Fluidextract of Ergot U. S. P. No. 636 is a standard preparation obtained from the Pharmacological Laboratory of the Bureau of Chemistry of the United States Department of Agriculture. This fluidextract

contains 0.050% of alkaloid in terms of ergotoxine phosphate or 0.0422% of ergotoxine base or 0.0522% of ergotamine base (1 mg. ergotoxine base = 1.3 mg.

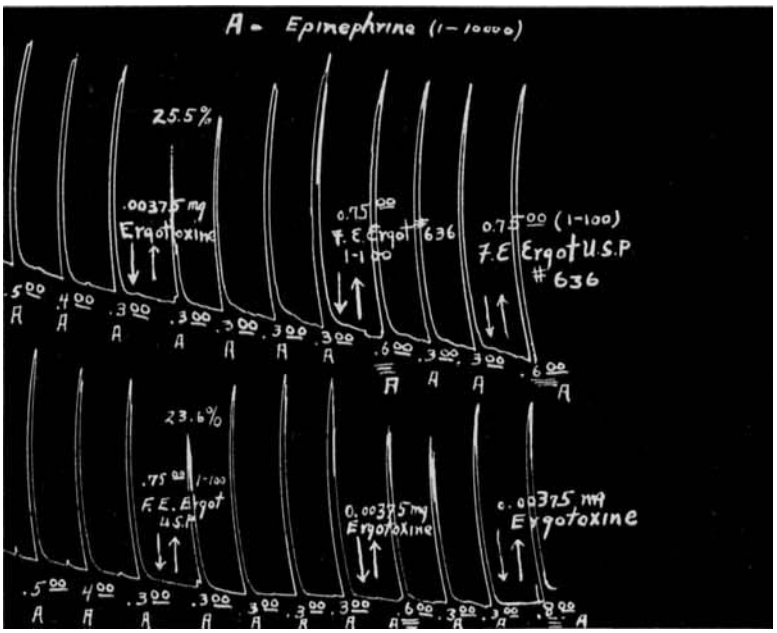

Fig. 2.—This tracing illustrates Methods B and C.

ergotamine base) as assayed by the above Methods A and B. However, by Method C the same Fluidextract of Ergot U. S. P. No. 636 assays 0.0375% of alkaloid in

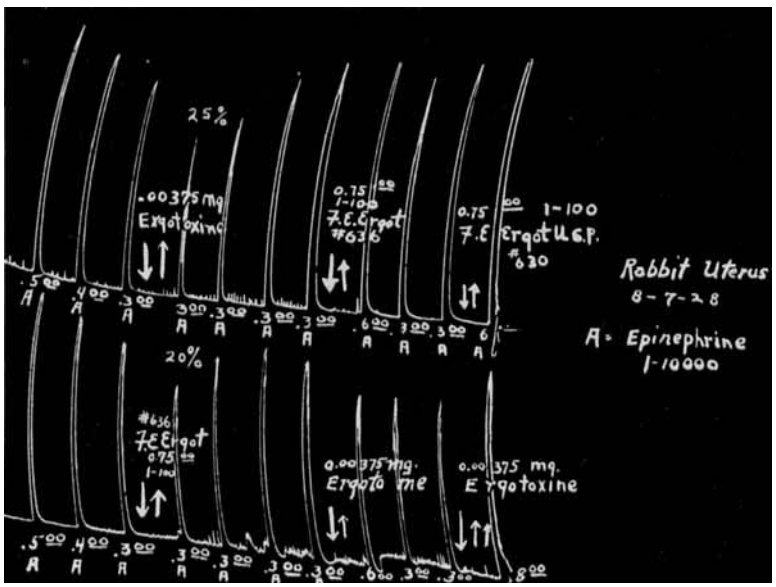

Fig. 3.—This tracing illustrates Methods B and C.

terms of ergotoxine phosphate, or 0.0316% in terms of ergotoxine base or 0.0410% of ergotamine base.

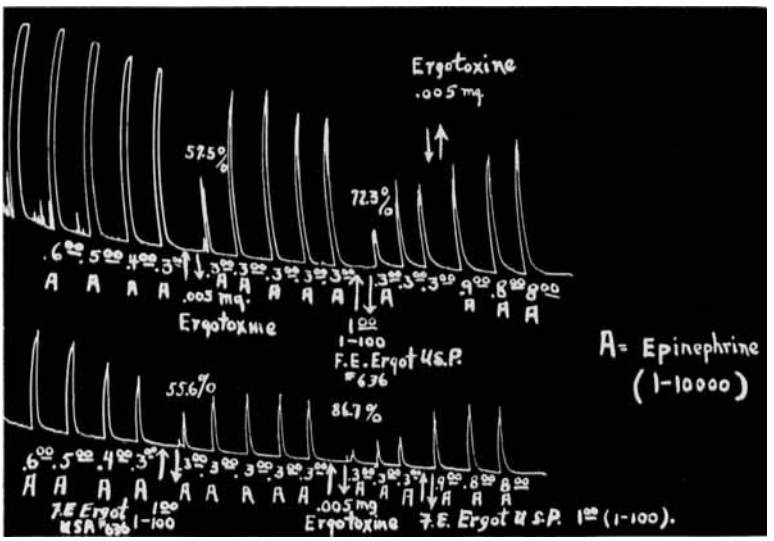

Fig. 4.—This tracing illustrates Methods B and C.

As stated previously the tests in the Tables I, II, III and IV were made by Methods A and B. Method C requires further study and will be reported in another article.

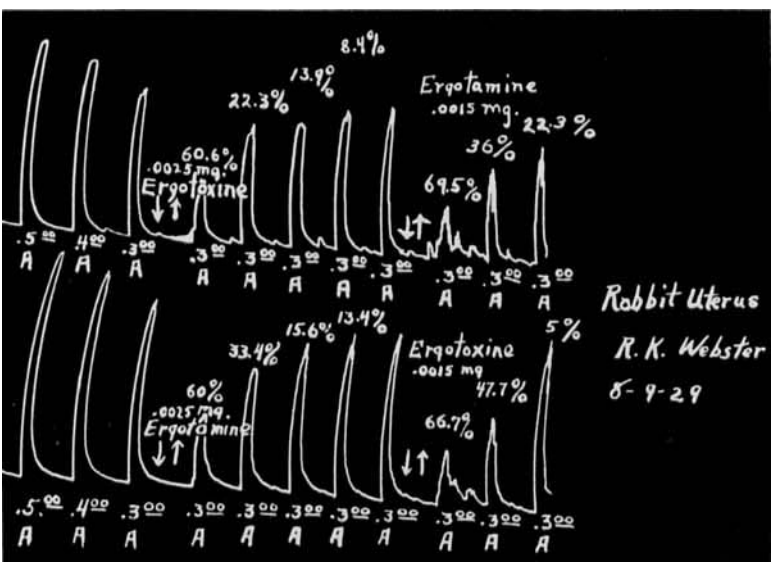

Fig. 5.—This tracing illustrates Method B.

7. SUMMARY.

1. The comparison of the U. S. P. Cock's Comb Method and the Broom and Clark Method gave similar results on a number of fluidextracts and solutions

or ergot. The Broom and Clark Method involves more skill and technique than the U. S. P. X. Some modifications of the Broom and Clark Method are being studied and further work is in progress.

2. The F. E. Ergot U. S. P. seems to be comparatively stabile, although further tests are necessary.

3. The solution of ergot for hypodermic use deteriorates rapidly.

4. A Fluidextract of Ergot was treated with various amounts of acid. The hydrogen-ion concentration seems to have some influence in controlling the deterioration and stabilization.

5. Further experiments are now in process to determine the value of the hydrogen-ion concentration factor.

6. Ergotoxine is distinctly stronger than ergotamine. The writer is much indebted to Dr. H. W. Cole for criticism of manuscript, to F. A. Whipple for hydrogen-ion concentration determinations, and to R. K. Webster, C. E. Powell and C. C. Hargreaves for assistance in making some of the tests.

EXPLANATION OF ILLUSTRATIONS.

Figure 1 represents a rabbit uterus (one fallopian tube cut in two equal parts). Gradually decreasing doses of epinephrine were injected, then to the upper muscle 1 cc. of 1–100 dilution of F. E. Ergot U. S. P. No. 636 was injected into the 100 cc. chamber, the ergot allowed to remain in contact with the muscle 5 minutes, then washed and the same amount of epinephrine is added as before ergot injection. The paralysis of ergot caused a 62.3% reduction or inhibition. The same muscle was given repeated injections of epinephrine. Note the recovery of the muscle to epinephrine stimulation. Two injections of 3 cc. (1–10,000) of epinephrine produced equal contractions. 1 cc. (1–100) F. E. Ergot U. S. P. No. 636 was again injected, 5 minutes' duration allowed, washed and 0.6 cc. of epinephrine added. This produced only 37.5% inhibition or reduction. Two cc. of 1–10,000 epinephrine was injected twice, 0.005 mg. of ergotoxine injected, which produced a 63.2% reduction or inhibition. The lower muscle received gradually decreasing amounts of epinephrine, then 0.0065 mg. of ergotoxine was added. This produced complete inhibition or paralysis to further 0.4 cc. (1–10,000) doses of epinephrine. Therefore, F. E. Ergot U. S. P. No. 636 contains 0.005 mg. of ergotoxine phosphate per 1 cc. (1–100) or 0.05% of ergotoxine phosphate according to the upper tracing or muscle.

Figure 2 represents a rabbit uterus (one fallopian tube cut in two equal parts). Gradually decreasing doses of epinephrine were injected, then to the upper muscle 0.00375 mg. of ergotoxine and the lower muscle 0.75 cc. 1–100 F. E. Ergot U. S. P. No. 636 were injected, 5 minutes' duration allowed, and washed. This produced a reduction of 25.5% with ergotoxine to the upper muscle. The lower tracing or muscle shows a 23.6% reduction or inhibition with the F. E. Ergot U. S. P. No. 636. Compare this with Fig. 1, which received 1 cc. (1–100) of F. E. Ergot U. S. P. No. 636 and 0.005 mg. of ergotoxine and gave 62.3% and 63.2% paralysis, respectively.

Repeated injections of epinephrine shows a gradually returning response. Following three injections of epinephrine 0.75 cc. (1–100) F. E. Ergot U. S. P. No. 636 and 0.00375 mg. of ergotoxine were injected, then washed after 5 minutes' duration, and then 0.6 cc. (1–10,000) of epinephrine or twice the dose of epinephrine as before ergot was injected. The upper tracing shows equal contractions, the lower tracing did not come up to the control—thus assuming that 0.00375 mg. of ergotoxine is stronger than 0.75 cc. 1–100 of F. E. Ergot. Later the same doses were given but to the lower muscle 0.8 cc. of epinephrine was injected which equaled the previous contraction. According to this tracing the F. E. Ergot U. S. P. No. 636 contains slightly less than 0.05% of ergotoxine phosphate.

Figure 3 represents the same rabbit uterus as Fig. 2, but is the other fallopian tube cut into two equal parts. The same treatment was applied to these muscles as in Fig. 2. Note the similarity of action as in Fig. 2. The lower tracing is not so reliable because the pointer seemed to scratch and the operator could not make adjustments without causing irregularities.

Figure 4 represents a rabbit uterus. Gradually decreasing doses of epinephrine were injected, which show a gradual decrease in contractions. To the upper muscle 0.005 mg. of ergotoxine and to the lower muscle 1 cc. 1–100 F. E. Ergot U. S. P. No. 636 were injected. This produced a reduction or inhibition of 57.5% and 55.6%, respectively. The same injections were later repeated only reversed (the upper receiving ergotoxine and the lower F. E. Ergot). This gave a reduction or inhibition of 72.3% and 86.7%, respectively. This tracing shows that 0.005 mg. of ergotoxine is slightly stronger than 1 cc. 1–100 F. E. Ergot U. S. P. No. 636.

Figure 5 represents a rabbit uterus (one fallopian tube cut into equal parts); 0.0025 mg. of ergotoxine and 0.0025 mg. of ergotamine produced inhibition or partial paralysis, 60.6% and 60%, respectively. Repeated equal-size doses of epinephrine show a gradual recovery, each succeeding repeating dose showing a decrease in inhibition or reduction. For ergotoxine 60.6%, 22.3%, 13.9% and 8.4% and finally back to the control.

For ergotamine 60%, 33.4%, 15.6%, 13.4% and finally almost to the control.

A second injection of these drugs with smaller doses of 0.0015 mg., but reversed, show a reduction or inhibition of 69.5%, 36% and 22.3% for ergotamine and 66.7%, 47.7% and 5% for ergotoxine. This tracing seems to show that ergotoxine is about equal to ergotamine, however, many tests similar to this show that ergotoxine is distinctly stronger than ergotamine.

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(9) Pattee and Nelson, *J. Pharmacol. & Exper. Therap.*, 36 (1929), 85.
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ABSTRACT OF DISCUSSION.

M. R. Thompson, commenting on the results of the experiments, said that the amines or water-soluble substances could occur in certain substances and might interfere greatly in the Cock's Comb or other methods.

E. E. Swanson stated that Pattee and Nelson, *J. Pharmacol. & Exper. Therap.*, 36 (1929), found that histamine inhibited the action of ergotamine. Also Dr. A. J. Clark, co-author of the Broom and Clark Method, observed that washing is necessary in order to prevent the antagonistic action of the amines. This has also been verified by the experiments of the authors, who found that two or three washings are sometimes necessary to abolish the amine antagonistic action.

M. R. Thompson further commented that certain investigators in this country have used this Broom and Clark Method and modified it in such a manner as to avoid washing after the administration of the ergot preparation, and then followed by the epinephrine. This technic cannot be used in preparations which are high in amines, and which greatly interfere with the activity. The effect will be one of low potency because the amines have a tendency to sensitize the uterus and the alkaloids will diminish the effect.

In regard to samples of ergotoxine, the results in their activity have been variable, whereas ergotamine samples do not vary.

James C. Munch remarked that Dr. A. J. Clark, in a personal interview, said that he found washing was necessary with uteri from certain breeds of rabbits.

E. F. Cook stated that it might be permissible to mention a new method just being proposed, an assay which gives some promise—that of Reynold and Carlson, which will be published in the *Journal of Physiology* in October. They use the living rabbit, determining the effect of the pituitary and also ergot on the uterus. They operate and draw out the uterus through the side of the rabbit, establishing a fistula. When the experiment is complete they make direct

observations of the contractions of the uterus. They discovered there was a certain period, possibly seven days during each month, when the uterus would not react, and so they were compelled to utilize the period when the uterus was responsive. They secured very definite readings from the contraction of the uterus.

F. O. Taylor requested information as to what acid was used for developing the p_H value of different samples tested for stability, and also if these samples were assayed chemically.

Mr. Swanson stated that no chemical test was tried.

ABSTRACTS OF PAPERS OF SCIENTIFIC SECTION, A. PH. A., 1929.

"Coal Tar Food Colors," by S. E. Owen.

Colors obtained in dying wool from acid and alkaline solutions recorded. Some general chemical tests are also given for differentiation of various colors.

"Amino Alcohols: 4. Reactions with Alkaloidal Reagents," by James C. Munch and W. H. Hartung.

Precipitation and color reactions of series of products homologous with ephedrine are reported.

"Amino Alcohols: 5. A Potentiometric Study of Certain Homologues of Ephedrine," by John C. Krantz, Jr., and W. H. Hartung.

A potentiometric study including the degrees of hydrolysis and association constants of certain homologs of ephedrine is reported.

"Ultraviolet Transmission of Liquids," by Ellery H. Harvey.

Transmission ultraviolet light measured by decomposition oxalic acid catalyzed by uranium salts. Tested large group of essential, fatty and crude oils as well as liquid chemicals. Application to storage in various wave-lengths of light discussed.

"Olive Oil—Fluorescence in Ultraviolet Light," by L. Deuble and R. E. Schoetzow.

The value of ultraviolet light as a means of detecting the presence of refined or treated olive oil in virgin olive oil is discussed.

"A Comparison of the Tissue Toxicity and Germicidal Power of Germicides," by Lester C. Himebaugh and Emil C. Fanto.

The toxicity of various germicides to living tissues grown outside the body has been compared with their germicidal action.

"Comments on U. S. P. X test for Rhapontic Rhubarb," by R. A. Konnerth and R. E. Schoetzow.

The unreliability of the U. S. P. test is pointed out and the more reliable method of the German Pharmacopœia is recommended.

"Digitalis Investigations. III. Digitalis Seed, Histology and Microchemistry," by Arno Viehoever and K. Shinohara.

Morphological, histological characteristics have been established. The presence and distribution of the active constituents was ascertained by microchemical methods. Digitalin and Digitonin are present in the endosperm and embryo. Digitalin occurs in larger amounts in the mature than in the immature seed.

"Pharmacognosy of Psyllium Seed," by Arno Viehoever and E. L. McLaughlin.

Morphological characteristics as well as histological have been established. A new method for the quantitative determination of the water-soluble mucilage in the outer seed coat has been devised. About 2.5 per cent of ash-free mucilage has been found in commercial seed as well as that grown under controlled conditions.

"Reactions between Formaldehyde and Hydrogen Peroxide and Quantitative Method Based Thereupon," by Arno Viehoever and K. Shinohara.

The various chemical reactions between Formaldehyde and Hydrogen Peroxide have been studied and a quantitative method of determination evolved.

"Digitalis Investigations. I. Effect of Digitoxin on the Heart of Daphnia Magna," by Arno Viehoever and A. McReyya.

Saturated aqueous solution increased tone of heart muscle and heart stopped in five to six hours. Toxic effect observed sooner in the presence of chloroform.

"Digitalis Investigations. II. Effect of Digitonin on the Heart of Daphnia," by Arno Viehoever and Manuel Tubis.

Saturated aqueous solution of digitonin behaved in general like saponin solution. A general slowing of the heart beat was observed, also slowing of antennae and increased peristalsis.
