of formaldehyde, a 1% solution of mercuric chloride, a saturated solution of arsenic trioxide, embalmed, unpreserved and exposed, or unpreserved and protected from the elements. Time is a negligible factor in the disappearance of the poison.

*3.* Lead is recovered to about the same extent as mercury when it is exposed to similar conditions.

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## VIII. THE STANDARDIZATION AND STABILIZATION OF ERGOT PREPARATIONS.*

BY EDWARD E. SWANSON, CLARENCE E. POWELL, ASA N. STEVENS AND E. H. STUART.

Since 1923, when Broom and Clark (1) first introduced the Isolated Rabbit Uterus Method, there has been some discussion as to which of the many methods give the more accurate results for the standardization of ergot preparations. To briefly summarize the literature, Burn (2), Linnell and Randle (3), Burn and Ellis (4), Nelson and Pattee (5), Pattee and Nelson (6), Thompson (7), and Swanson (8) apparently agree that the Epinephrine-Reversal Uterus Method (Broom and Clark) is equal to or more accurate than the U. S. P. Cock's Comb Method in determining the potency of ergot. Thompson (7) recommended that the Epinephrine-Reversal Method be adopted as the official method in the next Pharmacopœia in place of the now official U. S. P. Cock's Comb Method for the biological assay of ergot and its preparations.

It is the purpose of the writers to report in this article further (1) comparative study of the Epinephrine-Reversal and Cock's Comb Methods, and (2) the $p_H$ or hydrogen-ion concentration in relation to deterioration and stabilization of ergot and its preparations.

### EPINEPHRINE-REVERSAL METHOD.

Pattee and Nelson (6) state that much valuable time can be saved by external examination of the vaginal orifice in the selection of a uterus. Long and Evans (9) investigated the œstrus cycle of various animals and found that the rabbit is an exception in that the ovum is present only at the time of copulation. Knude and Proud (10) observed that there is no regularity in the appearance or disappearance of the different types of epithelial cells or leucocytes in the lumen of the vagina of normal rabbits. Our own experience showed that by vaginal smears studied under a microscope no definite œstrus cycle could be determined in rabbits.

The selection of a muscle with the Epinephrine-Reversal Method as previously reported requires some care and experience. The segregation of young female rabbits until they reach maturity (2.5 Kg. to 3 Kg.) is helpful in obtaining a suitable uterus. This also eliminates the influence of pregnancy, multiparous parturition and postpartum factors. The size of the individual uterus, regardless of the age of the rabbit, is variable. However, this is not so great

* Scientific Section, A. PH. A., Miami meeting, 1931.

when the rabbits are segregated. A uterus with strong active normal rhythmic contractions is usually too rapid in its contractions when stimulated with epinephrine and is not in our findings considered so reliable.

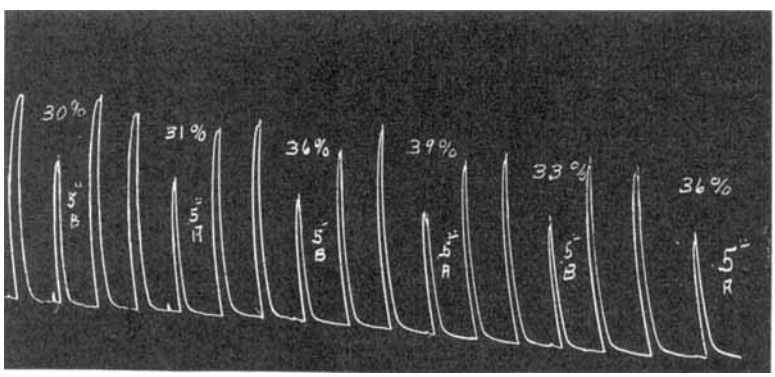
By the segregation of young rabbits allowed to reach maturity (2.5 Kg. to 3 Kg.) and careful external examination of the vaginal orifice, there is no difficulty in obtaining a suitable uterus.

TECHNIQUE OF ASSAYING BY THE EPINEPHRINE-REVERSAL METHOD.

Having selected a suitable uterus, the following three methods of technique have been studied. These methods have been previously reported in a preliminary way by one of us (Swanson (8)). They are here again discussed with added data and technical findings.

*A*. *Complete paralysis* of the uterus by ergot.
*B*. *Partial paralysis* of the uterus.
*C*. *The ratio of epinephrine stimulation to ergot paralysis.*

*Method A*.—A rabbit is killed and the uterus is excised. The excised uterus is divided into one-cm. pieces longitudinally in the plane of the mesenteric attachment. With virgin or




A = F.E. Ergot U.S.P. #2
B = F.E. Ergot U.S.P. #2 aerated 20 hours

Figure V

small rabbits, it is best to use whole segments of the uterus. Two pieces are mounted together in a bath of Ringer fluid.

The reaction consists in introducing epinephrine and observing the height of contraction; after washing out the epinephrine the ergot preparation is introduced and is left in for five minutes. The fluid is then changed and five minutes later epinephrine (same dose as above) is again introduced, and the effect observed. If epinephrine still produces a contraction the experiment is repeated with an increased concentration of the ergot. The approximate strength of the preparation can thus be determined fairly rapidly, and then the experiments are repeated on fresh uterine strips, the end-point desired being the concentration of the preparation which in a single dose almost completely abolishes the epinephrine response.

*Method B*.—Partial paralysis involves the use of small doses of ergot (both standard and unknown) that will only partially inhibit the epinephrine stimulation (20% to 50% inhibition). 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 at least twice, and five minutes later the same amount of epinephrine is injected as before ergot application. The amount of inhibition is measured, compared with the control and the reduction calculated. Injections of epinephrine are repeated until the contractions of the uterus return to constant

height with the same amount of epinephrine. The recovery of a uterus to constant height varies from 20 to 60 minutes. 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

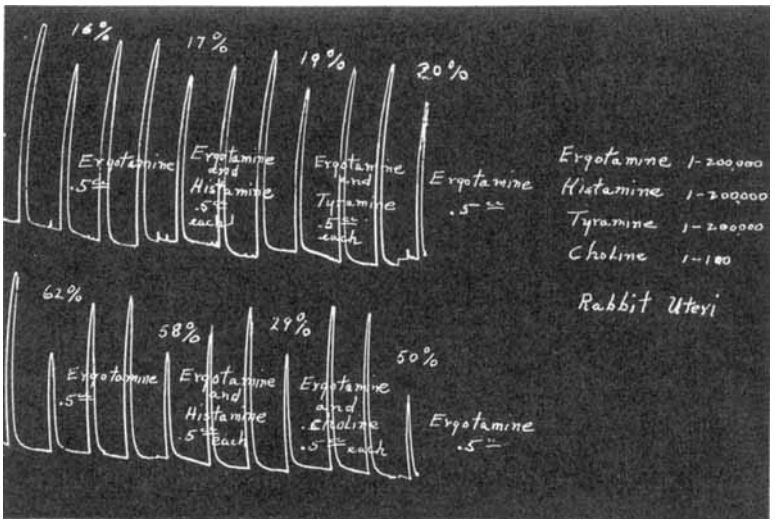



Figure I

of several doses of ergot on the same uterus. By alternating the unknown and standard on the same muscle one can obtain fairly accurate comparisons as shown in Figure V.

*Method C.*—This technique determines the amount of paralysis in terms of epinephrine, that is, following the ergot paralysis the amount of epinephrine injected is increased until contractions equal in height the contraction of epinephrine before ergot was injected. Thus the amount

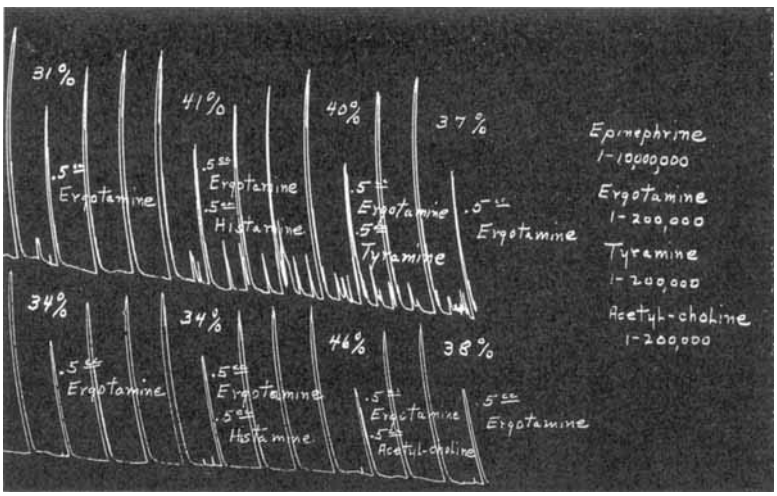



Figure II

of increase in epinephrine depends upon the amount of ergot injected or the degree of paralysis caused by ergot.

All of the above three methods will give accurate results. More than five hundred samples of ergot have been assayed by these three methods of technique.

The results show that *Method B is the more practical requiring less time and the inhibiting dose of ergot more easily controlled.*

THE EFFECT OF HISTAMINE, TYRAMINE AND CHOLINES ON ERGOT INHIBITION.

Pattee and Nelson (6) have shown that histamine alters considerably, if not repeatedly washed, the ergot inhibition by augmenting the epinephrine contrac-

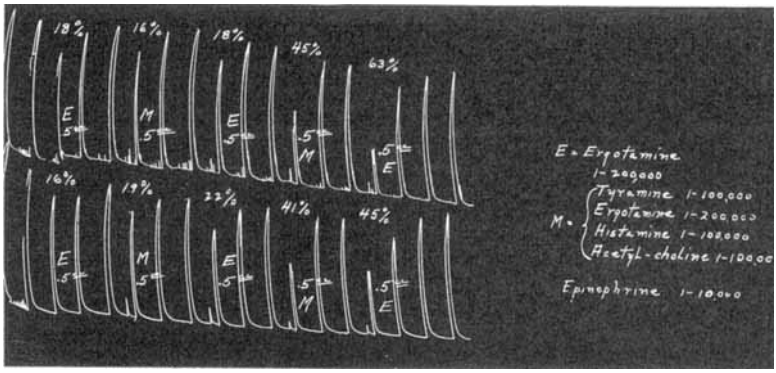



Figure III

tions. This, however, was not observed if several washings were made after ergot application. Our own findings confirm this observation. We have extended this investigation with tyramine, choline and acetyl-choline which are, like histamine, known constituents in the ergot drug. As shown in Figures I, II and III, doses of ergotamine tartrate 1–40,000,000 produce distinct inhibition. Histamine, tyra-

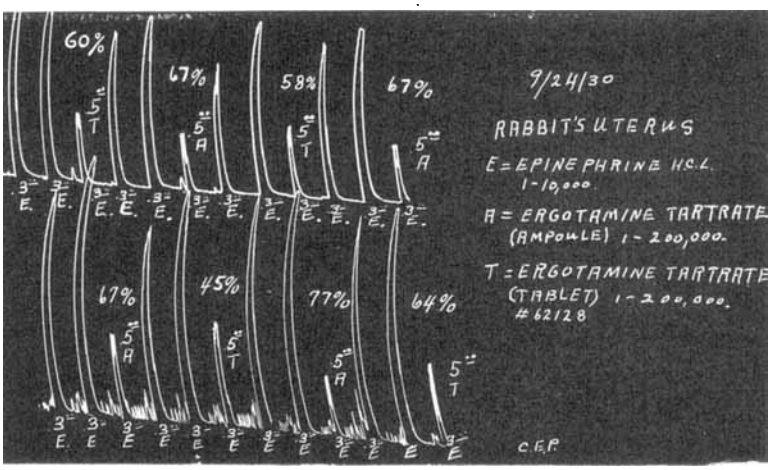



Figure IV

mine and acetyl-choline each with dilutions of 1–20,000,000 to 1–40,000,000, given alone or with ergotamine tartrate, allowed to act for five minutes, produce no inhibition or prevents the inhibiting effect of ergotamine. These figures are based on the assumption that the ergot drug contains the same or twice as much of each of the amines as that of the alkaloids. Thus an ergot drug containing 0.05% to 0.10% of

the amines apparently has no synergistic or antagonistic effect on the physiological assay of the ergot alkaloid.

### COMPARATIVE DATA WITH ERGOT BY THE EPINEPHRINE-REVERSAL UTERI AND COCK'S COMB METHOD.

Broom and Clark (1), Burn (2), Burn and Ellis (4), Linell and Randle (3), Pattee and Nelson (5,6), Prybill and Maurer (11), Thompson (7), and Swanson (8), have already shown that the Epinephrine-Reversal and Cock's Comb Methods gave correlative results in determining the potency of ergot. Tables I and II show further confirmative data that the Epinephrine-Reversal Method is in close agreement with the Cock's Comb Method. In average figures the Epinephrine-Reversal Method gives slightly higher potency values for ergot than the Cock's Comb Method.

TABLE I.—COMPARATIVE CRUDE DRUG ERGOT ASSAY.

Error ± 10.

| Crude Drug No. | Date of Assay. | Reversal Uteri Method, Per Cent. | Cock's Comb Method, Per Cent. |
|---|---|---|---|
| 1 | 12- 3-29 | 100 | 100 |
| 2 | 4-16-30 | 100 | 100 |
| 3 | 6-16-30 | 100 | 100 |
| 4 | 6-23-30 | 100 | 80 |
| 5 | 6-23-30 | 100 | 90 |
| 6 | 6-24-30 | 75 | 60 |
| 7 | 6-30-30 | 100 | 100 |
| 8 | 6-30-30 | 100 | 100 |
| 9 | 6-30-30 | 100 | 100 |
| 10 | 10-24-30 | 100 | 120 |
| 11 | 10-24-30 | 100 | 100 |
| 12 | 11-14-30 | 100 | 100 |
| 13 | 11-18-30 | 50 | −50 |
| 14 | 11-18-30 | 50 | 70 |
| 15 | 11-19-30 | 50 | 50 to 100 |
| 16 | 11-19-30 | 25 | 25 to 50 |
| 17 | 11-20-30 | 100 | 110 |
| 18 | 12-30-30 | 100 | 100 |
| 19 | 12-30-30 | 100 | 90 |
| 20 | 12-30-30 | 100 | 100 |
| 21 | 1-19-31 | 100 | 100 |
| 22 | 1-20-31 | 100 | 100 |
| 23 | 1-21-31 | 100 | 100 |
| 24 | 2- 2-31 | 100 | 140 |
| 25 | 2- 2-31 | 50 | 75+ |
| 26 | 2-20-31 | 75 | 100 |
| 27 | 3- 4-31 | 100 | 100 |
| 28 | 3- 4-31 | 100 | 100+ |
| 29 | 4-24-31 | 150 | 100+ |
| 30 | 4-24-31 | 100 | 100+ |
| 31 | 5-11-31 | 100 | 100 |
| 32 | 5-11-31 | 100 | 100+ |
| 33 | 6- 2-31 | 50 | −50 |

It is our opinion as the result of over five hundred tests within the last three years, that either method of assay can be used to determine the alkaloidal content of ergot preparations. Either method cannot be used with accuracy without some

experience. We also find that by the careful segregation of young females allowed to reach maturity, and with experience in selecting a suitable uterus, the Epinephrine-Reversal Method is less costly and is more accurate in determining the alkaloidal content of ergot. Therefore, *it is recommended that the Epinephrine-Reversal Method be considered as a supplementary assay to the Cock's Comb Method in determining the potency of ergot preparations.*

TABLE II.—COMPARATIVE F. E. ERGOT U. S. P. ASSAY.

Error ± 10.

| Date. | F. E. Ergot U. S. P., Sample No. | Cock's Comb Method, Per Cent Activity. | Reversal Uteri Method (Broom and Clark). Alkaloidal content calculated. | Per cent activity. |
|---|---|---|---|---|
| 11–14–29 | 1 | 80 | 0.0500 | 90 |
| 11–15–29 | 2 | 90 | 0.0475 | 95 |
| 11–22–29 | 3 | 50 | 0.0325 | 65 |
| 11–26–29 | 4 | 110 | 0.0550 | 110 |
| 1–17–30 | 5 | 120 | 0.0650 | 130 |
| 2– 5–30 | 6 | 150 | 0.1000 | 200 |
| 2– 4–30 | 7 | 105 | 0.0500 | 100 |
| 7–22–30 | 8 | 100 | 0.0500 | 100 |
| 7–23–30 | 9 | 100 | 0.0500 | 100 |
| 8–22–30 | 10 | 100 | 0.0500 | 100 |
| 8–22–30 | 11 | 50 | 0.0333 | $66^2/_3$ |
| 1–13–31 | 12 | 100 | 0.0500 | 100 |
| 1–20–31 | 13 | 90 | 0.0500 | 100 |
| 2–20–31 | 14 | 100 | 0.0550 | 110 |
| 3–25–31 | 15 | 100 | 0.0500 | 100 |
| 4–20–31 | 16 | 40 | 0.0250 | 50 |
| 4–20–31 | 17 | 60 | 0.0400 | 80 |
| 6– 2–31 | 18 | 40 | 0.0250 | 50 |
| 6–15–31 | 19 | 95 | 0.0500 | 100 |

A CHEMICAL AND BIOLOGICAL COMPARISON.

As shown in Table III a Fluidextract of Ergot was prepared by percolating with neutral 50% alcohol. The $p_H$ of this fluidextract without acid was 5.77 and assayed 100% by the Epinephrine-Reversal and U. S. P. Cock's Comb Methods. This fluidextract was divided into twenty-two (22) parts. As shown in Table III various quantities of tartaric acid, hydrochloric acid and sulphuric acid were added to nineteen (19) of the twenty-two (22) samples. Samples Nos. 1, 8, 15 are the same and represent the original fluidextract with neutral alcohol menstruum. The hydrogen-ion concentration or $p_H$ of these three samples was 5.77, 5.79 and 5.80, respectively. These $p_H$ findings are considered reliable checks inasmuch as the $p_H$ operator did not know that they were samples of the original fluidextract. Table III shows the hydrogen-ion concentration ($p_H$) of all the samples.

Three to six months later these samples were assayed by the Epinephrine-Reversal Method and Smith's (15) Chemical Method. The chemical method (Smith's colorimetric method) was used with the following modifications. Five cubic centimeters of the Fluidextract of Ergot were placed in a small separatory funnel and placed on a steam-bath in a current of air to evaporate the alcohol. After evaporation 50 cc. of distilled water and 2 cc. of ammonium hydroxide (1 + 1) were added to the separatory funnel. The mixture was shaken several times. This solution was then run into a Watkin's Extraction Apparatus and extracted with ether for four hours. The ether extract was then transferred to a separatory funnel and washed with two 25-cc. portions of

distilled water, which removed most of the yellow pigment present in the ether solution. Shake the ether solution with three separate (10 cc., 10 cc. and 5 cc.) portions of 1% tartaric acid. Evaporate the tartrate solution on a water-bath to remove ether and make up to 40 cc. Two cubic centimeters of this solution and one cubic centimeter of *p*-dimethyl-aminobenzaldehyde solution are mixed. The color is allowed to develop and compared with color solutions of the standard ergotamine tartrate.

As shown in Table III and Chart III, the chemical method gives correlative results with the Epinephrine-Reversal Method. As a whole the chemical results are slightly lower than the biological. The biological assays were made the third, fourth, fifth and sixth months. The chemical assays were carried out during the fifth and sixth months.

TABLE III.—A CHEMICAL AND BIOLOGICAL COMPARISON.

This table represents a Fluidextract of Ergot divided into 22 parts and various amounts of acids added. The original assay was 100% by the Cock's Comb and Reversal Uteri Methods.

| Fluidextract of Ergot, Sample No. | Date Made. | Acid. | $p_H$. | Chemical Method (Smith). Date of assay. | Chemical Method (Smith). Activity. | Reversal Uteri Method (Broom and Clark). Date of assay. | Reversal Uteri Method (Broom and Clark). Activity. |
|---|---|---|---|---|---|---|---|
| 1 | 1-3-30 | None | 5.77 | 6-23-31 | 40% | 5-27-31 | 40% |
| 2 | 1-3-30 | Tartaric | 5.28 | ..... | .. | ..... | .. |
| 3 | 1-3-30 | Tartaric | 5.13 | 6-25-31 | 20% | 5-29-31 | 30% |
| 4 | 1-3-30 | Tartaric | 4.87 | 6-25-31 | 40% | 5-26-31 | 50% |
| 5 | 1-3-30 | Tartaric | 4.67 | 6-23-31 | 50% | 5-11-31 | 60% |
| 6 | 1-3-30 | Tartaric | 4.26 | 6-23-31 | 50% | 5-25-31 | 50% |
| 7 | 1-3-30 | Tartaric | 3.89 | 6-23-31 | 90% | 5-29-31 | 100% |
| 8 | 1-3-30 | None | 5.79 | 6-23-31 | 40% | 5-27-31 | 40% |
| 9 | 1-3-30 | HCl | 4.71 | ..... | .. | 6- 8-31 | 30% |
| 10 | 1-3-30 | HCl | 4.48 | 6-29-31 | 30% | 6-12-31 | 37.5% |
| 11 | 1-3-30 | HCl | 4.11 | 6-29-31 | 64% | 6- 9-31 | 75% |
| 12 | 1-3-30 | HCl | 3.55 | 6-29-31 | 80% | 6-15-31 | 90% |
| 13 | 1-3-30 | HCl | 3.04 | 6-30-31 | 92% | 6-10-31 | 100% |
| 14 | 1-3-30 | HCl | 2.58 | 6-30-31 | 74% | 6- 9-31 | 85% |
| 15 | 1-3-30 | None | 5.80 | 6-23-31 | 40% | 4-26-31 | 40% |
| 16 | 1-3-30 | Sulphuric | 4.72 | 6- 2-31 | 20% | 4- 6-31 | 30% |
| 17 | 1-3-30 | Sulphuric | 4.51 | 5-15-31 | 28.8% | 3-29-31 | 35% |
| 18 | 1-3-30 | Sulphuric | 4.10 | 5-15-31 | 40% | 3- 6-31 | 50% |
| 19 | 1-3-30 | Sulphuric | 3.45 | 5-15-31 | 40% | 3-23-31 | 50% |
| 20 | 1-3-30 | Sulphuric | 2.95 | 5-15-31 | 40% | 3-22-31 | 50% |
| 21 | 1-3-30 | Sulphuric | 2.60 | 5-15-31 | 24% | 3-28-31 | 30% |
| 22 | 1-3-30 | Sulphuric | 2.25 | 5-15-31 | 20% | 3-30-31 | 30% |

In regard to the hydrogen-ion concentration ($p_H$) of these samples, *the $p_H$ appears to influence the stability of the alkaloids for at least six months, providing they are not exposed to air.* The samples close to a $p_H$ of 3.00 show little deterioration with tartaric acid and hydrochloric acid. The samples with sulphuric acid show only 50% activity in three months at a $p_H$ of around 3.00. We can give no explanation as to why the samples with sulphuric acid show less activity than with tartaric acid and hydrochloric acid. Further data is necessary on the Chemical Method which will be reported later.

## THE EXTRACTION OF ERGOT DRUG BY NEUTRAL ALCOHOL AND ACID-ALCOHOL MENSTRUA.

Linnell and Randle (3) found that a series of fluidextracts, prepared with acid menstruum, contained considerably more of the alkaloid than when prepared with dilute neutral alcohol menstruum. These authors also observed that the presence

of alcohol as a solvent is essential, that a weaker alcohol solvent than 50% is unadvisable, and that a stronger solvent does not seem to be warranted.

Our findings agree with Linnell and Randle (3) in that an acid alcohol menstruum, particularly tartaric acid and hydrochloric acid, is more efficient than a neutral alcoholic menstruum in extracting the alkaloids of ergot. The extraction efficiency of this alcoholic acid menstruum, however, is dependent on the alcoholic percentage of the acid menstruum, as shown in the following table:

| Crude Ergot Drug Number. | $p_H$ of Crude Drug. | Alcohol Percentage. | Kind of Acid 1%. | Epinephrine-Reversal Broom & Clark %. | Chemical Method (Smith) %. |
|---|---|---|---|---|---|
| 1 | 6.2 | 40 | None | 90 | ... |
| .. | ... | 40 | Hydrochloric | 100 | ... |
| .. | ... | 40 | Tartaric | 100 | ... |
| .. | ... | 50 | None | 100 | ... |
| .. | ... | 50 | Hydrochloric | 110 | ... |
| .. | ... | 50 | Tartaric | 110 | ... |
| .. | ... | 60 | None | 110 | ... |
| .. | ... | 60 | Hydrochloric | 140 | ... |
| .. | ... | 60 | Tartaric | 150 | ... |
| .. | ... | 80 | None | 125 | ... |
| .. | ... | 80 | Hydrochloric | 180 | ... |
| .. | ... | 80 | Tartaric | 175 | ... |
| .. | ... | 95 | None | 120 | ... |
| .. | ... | 95 | Hydrochloric | 150 | ... |
| .. | ... | 95 | Tartaric | 140 | ... |
| 2 | 5.5 | 50 | None | 110 | 100 |
| .. | ... | 50 | Hydrochloric | 100 | 110 |
| .. | ... | $66^2/_3$ | None | 108 | 95 |
| .. | ... | $66^2/_3$ | Hydrochloric | 133 | 125 |
| .. | ... | 75 | None | 106 | 110 |
| .. | ... | 75 | Hydrochloric | 140 | 150 |
| .. | ... | 85 | None | 130 | 140 |
| .. | ... | 85 | Hydrochloric | 150 | 160 |

As shown in the above table, menstrua of neutral alcohol 40%, 50%, 60%, $66^2/_3$% and 75% appear to be of the same efficiency as solvents for the extraction of the alkaloids. Menstrua of 80%, 85% and 95% neutral alcohol are apparently better solvents than the lower percentages of alcohol. The presence of an acid (tartaric acid or hydrochloric acid) in 40% and 50% alcohol menstrua does not materially increase their solvent properties. The extraction value of these acid-alcohol solvents, is dependent on the alcoholic percentage of the acid menstruum. The extraction efficiency of a neutral alcoholic menstruum is influenced by the hydrogen-ion concentration ($p_H$) of the crude ergot drug, an ergot drug with a $p_H$ of 5.5 being more easily extracted than an ergot drug with a $p_H$ of 6.00 by a neutral alcoholic menstruum.

### THE HYDROGEN-ION CONCENTRATION FACTOR.

In a previous study we reported that the hydrogen-ion concentration appears to have some influence in controlling the deterioration and stabilization of ergot preparations. Swanson (8) and Wokes and Elphick (12) have found that "percolation of ergot with neutral 50% alcohol menstruum, the extraction efficiency is greatly affected by the degree of acidity of the ergot drug, due to the phosphate and

other buffering substances which it contains. With the more acid ergots ($p_H$ below 5.5) neutral alcohol menstruum may extract the ergot alkaloids almost completely as it is extracted by acidified (HCl or tartaric) alcohol. With less acid ergots ($p_H$ above 6.0) neutral alcohol may extract less than half the amount of alkaloids than that taken out by acidified alcohol. If this $p_H$ is to be maintained at the optimum point for extraction and stability, as suggested by some workers, it will be necessary to employ a fixed proportion of acid, but control will be necessary by means of $p_H$ determinations." Wokes and Elphick (12) further found that the defatting of ergot increases the efficiency of extraction of both neutral and acidified alcohol. Wokes (13) and Wokes and Elphick (14) reported that liquid extracts of ergot and solutions of ergotoxine phosphate deteriorate rapidly, the deterioration being greater at room temperature and at incubation temperature. Thompson (7) showed that deterioration of the alkaloidal activity of Fluidextract of Ergot U. S. P. X was not as rapid as reported by Wokes (13) provided that the preparation is protected from excessive exposure to air. Previously one of us (Swanson (8)) reported on a Fluidextract of Ergot U. S. P. with various hydrogen-ion concentrations (see Table II and Chart I, JOUR. A. PH. A., 28 (1929), 1130–1131. This series was again assayed at the end of the third year. During the third year these samples were frequently exposed to air. The deterioration now shows a different curve from that reported previously. There is a distinct deterioration in the whole series; however, the samples with a $p_H$ around 3.00 show the least deterioration (see Table IV and Chart I).

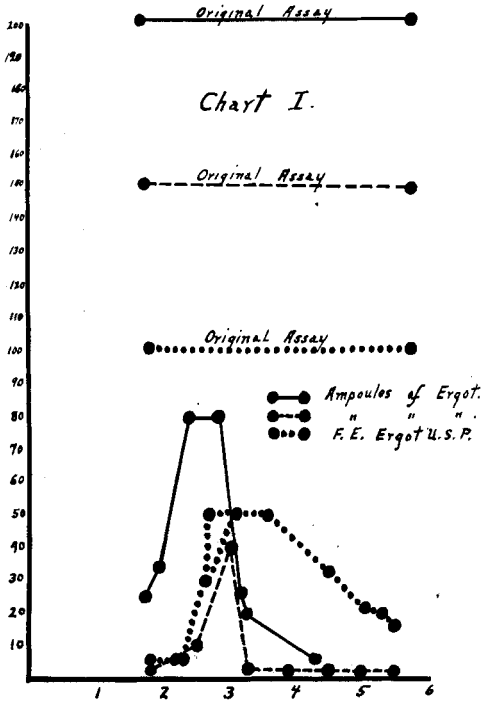



TABLE IV.—SAMPLE OF F. E. ERGOT WITH VARIOUS $p_H$ RANGE ASSAYED AFTER THREEE YEARS' AGING.

| F. E. Ergot Sample No. | $p_H$. | Cock's Comb Method (Per Cent Activity.) | Reversal Uteri Method (Broom and Clark). Alkaloidal content calculated. | Activity. |
|---|---|---|---|---|
| 1 | 5.35 | Less than 10% | 0.0075% | 15% |
| 2 | 4.95 | Less than 10% | 0.010% | 20% |
| 3 | 4.51 | Less than 10% | 0.011% | 22% |
| 4 | 3.77 | 20% | 0.015% | 30% |
| 5 | 3.21 | 30% | 0.025% | 50% |
| 6 | 2.69 | 30% | 0.025% | 50% |
| 7 | 2.26 | Less than 20% | 0.015% | 30% ? |
| 8 | 1.90 | Less than 10% | −0.005% | −10% |

Two preparations of solution of ergot for hypodermic use were prepared. As shown in Tables V and VI and Chart I there is a marked deterioration of all the samples. These two series were frequently exposed to air during the aging period. The samples around a $p_H$ of 3.00 show the least deterioration. The original assays of the two preparations when made were 150% and 200%, respectively.

The effect of aeration has also been partially studied. Four samples of Fluidextract of Ergot U. S. P. made from four different lots of ergot were selected. A part of each sample (200 cc.) was placed in eight-ounce amber-colored bottles. Air was passed through by a glass tube reaching to the bottom of the bottle. These samples were aerated for 12 hours, 20 hours, 48 hours and 72 hours. The results are as follows:

| Number of F. E. Ergot Sample. | Number of Hours Aerated. | $p_H$. | Epinephrine-Reversal Method. Before Aeration. | After Aeration. | Total Loss by Aeration. | Chemical Method. Before Aeration. | After Aeration. | Total Loss by Aeration. |
|---|---|---|---|---|---|---|---|---|
| 1 | 12 hrs. | 3.00 | ... | ... | ... | 100% | 100% | None |
| 2 | 20 hrs. | 2.70 | 100% | 90% | 10% | 100% | 87% | 13% |
| 3 | 48 hrs. | 2.80 | 100% | 80% | 20% | 120% | 104% | 16% |
| 4 | 72 hrs. | 2.96 | 110% | 110% | None | 100% | 100% | None |

As shown in the above table, the aeration of Fluidextract of Ergot with a $p_H$ of 3.00 is apparently little affected in its potency. A fluidextract with a $p_H$ of 2.70 and 2.80 show a 10% to 20% loss in activity when aerated for 20 hours to 48 hours.

All Fluidextracts of Ergot when exposed to excessivly high temperature, regardless of the $p_H$, deteriorate rapidly. Thus the hydrogen-ion concentration factor apparently has no influence in preventing the deterioration of the alkaloids of ergot when subjected to excessive heat or high temperature.

As shown in Chart II the hydrogen-ion concentration for some Fluidextracts of Ergot appears to have no influence in preventing deterioration. Fluidextract of Ergot No. 1, as shown in

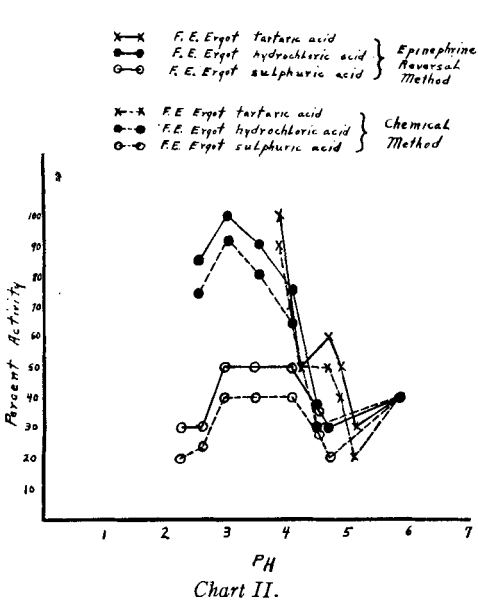



Chart II.

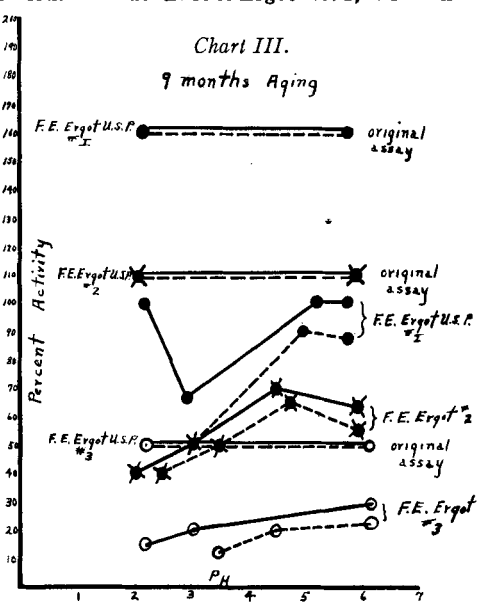



Chart II, originally assayed 160% by the Cock's Comb and Epinephrine-Reversal Methods. To various samples of this lot hydrochloric acid was added, thus varying the $p_H$ from 4.70 to 2.20. These samples, after nine months' aging, were again assayed by the two biological methods. Fluidextracts of Ergot Nos. 2 and 3, as shown in Chart II, were made from two different lots of ergot drug. After nine months' aging the samples were again assayed.

All three of the fluidextracts show a distinct deterioration regardless of the hydrogen-ion concentration. The various hydrogen-ion concentrations apparently had no influence in preventing the deterioration.

TABLE V.—ORIGINAL ASSAY = 150% SOLUTION OF ERGOT FOR HYPODERMIC USE.

| Sample No. | Date Made. | $p_H$. | Date Assayed. | Broom and Clark (Per Cent.) |
|---|---|---|---|---|
| 1 | 3-11-29 | 5.50 | 3-10-30 | 1% |
| 2 | 3-11-29 | 4.50 | 3-11-30 | 1.25% |
| 3 | 3-11-29 | 3.90 | 3-12-30 | 1.66⅔% |
| 4 | 3-11-29 | 3.30 | 3-14-30 | 2.5% |
| 5 | 3-11-29 | 3.00 | 3-18-30 | 40.0% |
| 6 | 3-11-29 | 2.50 | 3-21-30 | 10.0% |
| 7 | 3-11-29 | 2.20 | 3-24-30 | 5.0% |
| 8 | 3-11-29 | 1.80 | 3-30-30 | 2.0% |

Considering the results as shown in Tables IV, V, VI and Charts I and II: (1) Not all Fluidextracts of Ergot are influenced by the hydrogen-ion concentration in preventing deterioration. (2) For some Fluidextracts of Ergot the hydrogen-ion concentration of around 3.00 appears

TABLE VI.—ORIGINAL ASSAY = 200% SOLUTION ERGOT FOR HYPODERMIC USE.

| Sample No. | Date Made. | $p_H$. | Date Assayed. | Broom and Clark (Per Cent.) |
|---|---|---|---|---|
| 1 | 1-16-29 | 4.30 | 9-15-29 | 5% |
| 2 | 1-16-29 | 3.20 | 9-16-29 | 17% |
| 3 | 1-16-29 | 2.90 | 9-20-29 | 80% |
| 4 | 1-16-29 | 2.40 | 10- 7-29 | 80% |
| 5 | 1-16-29 | 1.90 | 10- 9-29 | 33⅓% |
| 6 | 1-16-29 | 1.75 | 10-11-29 | 25% |

to be the critical point where there is the least deterioration. (3) The passing of a current of air through a fluidextract appears to be influenced by the hydrogen-ion concentration in preventing deterioration. (4) The hydrogen-ion concentration appears to have no influence on a fluidextract if frequently exposed to air or subjected to excess heat or temperature.

(*To be continued*)

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# TRANSPARENT LIFE STUDIES.*

## 2. EFFECT OF STRYCHNINE UPON DAPHNIA.[1]

BY ARNO VIEHOEVER AND ANNA SCHWENK MIKURIYA.

The remarkable suitability of certain transparent organisms as Daphnia, an ideal representative of transparent life (1), for purpose of demonstration of major life functions, namely, the action of the muscular, glandular and nervous system, has been pointed out before by one of us (2). Equally the use of this or other transparent animals for testing drugs was suggested. To demonstrate that the effect of stimulants and depressants and other therapeutic agencies can be quantitatively checked, many experiments were carried out, others are under way. The results obtained with a soluble salt of the alkaloid strychnine on Daphnia magna will be first recorded here.

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* From the Biochemical Research Laboratories of the Philadelphia College of Pharmacy and Science. The studies have had the initial support of the AMERICAN PHARMACEUTICAL ASSOCIATION and are continued with the generous help of Dr. Kilmer, New Brunswick, supporting the Kilmer Fellowship.

[1] Scientific Section, A. PH. A., Miami meeting, 1931.