SCIENTIFIC SECTION

VIII. THE STANDARDIZATION AND STABILIZATION OF ERGOT PREPARATIONS.

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(*Continued from page 239, March 1932*)

EXPERIMENTAL DATA ON THE ALKALOIDS OF ERGOT.

Considering the above data and the reported work of others, the question of deterioration and stability of the ergot alkaloids is indeed a complicated problem. Stoll and Rothlin (18), Wokes (13), and Thompson (7) have already reported evidence showing that exposure to air or oxidation and increased temperature destroys the alkaloids of ergot. Swanson (8) observed that the hydrogen-ion concentration may have some influence on the stability of the alkaloids. In this report we show that the hydrogen-ion concentration still appears to have some control in the stability of the alkaloids of ergot.

There are many factors that may have some part in affecting the decomposition of the alkaloids of ergot. The acidity of the ergot drug, the process of defatting, the effect of temperature changes, the effect of oxidation or exposure to air, and the action of the amines and buffer salts which are naturally found in the drug, all appear to have some influence on the stability of the alkaloids.

Experimental evidence show that the ergot alkloids are the constituents which are specific for the drug and responsible for the desired clinical effects of its preparations. The amines in ergot are considered to be non-specific. Spiro and Stoll (16), Forst and Weese (17), and Thompson (7) observed that these non-specific amines are variable in the crude drug. The amines that have been identified in the drug are histamine, acetyl-choline and other cholines, tyramine, agmatine, isoamylamine and guanido-butylamine. There appears to be no available data as to the amount of these amines in the drug. Thompson (7) by his histamine guinea pig method and by the depressor method determined the amine content of ergot in terms of histamine. Thompson (7) found that various commercial crude ergot samples contained 0.012% to 0.15% of amines calculated in terms of histamine. He also suggests that in preparing the Fluidextract of Ergot the amines be removed by percolation with 5% sodium bicarbonate solution.

We are now studying the effects of various acids, hydrogen-ion concentration, various buffer salts and the various amines (histamine, tyramine and choline) on an alcoholic solution of the pure crystals of ergotamine tartrate (Sandoz) and ergotoxine salts.

As shown in Table VII ergotamine tartrate 0.05% was dissolved in 40% alcohol, 0.5% KH_2PO_4 was added and divided into five equal parts. To each part was added sufficient $M/5$ HCl to give the various hydrogen-ion concentrations. Each sample was aerated for 48 hours to 72 hours by passing a current of air through the solutions. After aeration each sample was assayed for total alkaloid by the Epinephrine-Reversal and Chemical (Smith's) Methods.

To samples Nos. 6, 7 and 8, 0.05% ergotamine tartrate was dissolved in 40% alcohol. $NaH_2PO_2H_2O$ 0.5% was added and the p_H adjusted to 2.20, 3.15 and 4.30, respectively, with $M/5$ HCl. Samples Nos. 9, 10 and 11 contain the same amount of ergotamine tartrate and the p_H adjusted with $M/5$ HCl. No buffer salts were added. These solutions were all aerated by a current of air. After aeration, and adjusted to the original volume with an alcoholic solution, the samples were assayed by the Epinephrine-Reversal (Broom and Clark) and the Chemical (Smith's) Methods.

As shown in Table VII the ergotamine solutions with hydrogen-ion concentrations of 4.20, 4.30 and 4.10 show a distinct loss in potency after 72 hours' aeration, particularly solutions Nos. 5 and 8. Apparently 0.5% of the buffers KH_2PO_4 and $NaH_2PO_2.H_2O$ did not aid in the prevention

of deterioration inasmuch as solution No. 11 adjusted with $M/5$ HCl to 4.10 alone shows only a 10% loss after aeration. Solutions Nos. 3, 4, 7 and 10 with hydrogen-ion concentrations of 2.90, 3.00, 3.15 and 2.92, respectively, show no loss of potency after 72 hours' aeration. Solutions Nos. 1, 6 and 9 with hydrogen-ion concentrations of 2.20, 2.20 and 2.10 show only a slight loss in potency after aeration; solution No. 1 with KH_2PO_4 and HCl showing a 10% loss by the Epinephrine-Reversal Method and no loss by the Chemical Method. Solution No. 6 with $NaH_2PO_2H_2O$ and HCl shows no loss by either method, and solution No. 9 a loss of 5% by the Biological Method and 10% loss by the Chemical Method after 72 hours' aeration.

TABLE VII.—AERATED SAMPLES OF ERGOTAMINE TARTRATE IN 40% ALCOHOL WITH VARIOUS BUFFERS AND p_H ADJUSTED WITH HCl.

				Epinephrine-Reversal Method.			Chemical Method (Smith's).		
No. of Sample.	Buffer 0.5%.	p_H Adjusted with HCl $M/5$.	Hours Aerated.	Before aeration.	After aeration.	Total loss by aeration.	Before aeration.	After aeration.	Total loss by aeration.
1	KH_2PO_4	2.20	72	100%	90%	10%	100%	100%	No loss
2	KH_2PO_4	2.90	48	100%	100%	No loss	...	...	....
3	KH_2PO_4	2.90	72	100%	100%	No loss	90%	90%	No loss
4	KH_2PO_4	3.00	72	90%	90%	No loss	80%	80%	No loss
5	KH_2PO_4	4.20	72	100%	50%	50%	100%	25%	75%
6	$NaH_2PO_2.H_2O$	2.20	72	100%	100%	No loss	100%	100%	No loss
7	$NaH_2PO_2.H_2O$	3.15	72	100%	100%	No loss	100%	100%	No loss
8	$NaH_2PO_2.H_2O$	4.30	72	100%	85%	15%	100%	75%	25%
9	None	2.10	72	100%	95%	5%	100%	90%	10%
10	None	2.92	72	100%	100%	No loss	100%	95%	5%
11	None	4.10	72	100%	90%	10%	100%	90%	10%

As shown in Table VII, a solution of pure ergotamine tartrate crystals in 40% alcohol with a *hydrogen-ion concentration of around 3.00 appears to be the critical point where there is the least deterioration.*

These experiments of various acids, buffers and reducing agents with the pure alkaloids of ergotamine and ergotoxine and the Fluidextract of Ergot is being continued and will be reported in a later article.

DISCUSSION.

Many workers with experience in the assay of ergot and its preparations apparently agree that the Epinephrine-Reversal Method is more accurate than the U. S. P. Cock's Comb Method. By the Epinephrine-Reversal Method, the amines (histamine, tyramine and cholines) in percentage equivalent to or twice that of the alkaloidal content of ergot, do not have synergistic or antagonistic effects on the inhibiting property of the alkaloids. By the U. S. P. Cock's Comb Method, there is experimental evidence that these amines apparently have some influence on the action of the ergot alkaloids.

The Chemical Method (Smith's) deserves further consideration. The results as shown in Table III show correlative results with the Epinephrine-Reversal Method. However, these results were obtained by comparison with the same ergotamine tartrate. We do not know whether all samples of ergotamine tartrate or ergotoxine phosphate or ergotoxine ethane-sulphonate in equivalent amounts of the base will give the same color reaction. Is ergotamine tartrate or ergotoxine phosphate a reliable standard for the Chemical Method (Smith's)? There is evidence that ergotamine tartrate contains more than one type of crystals.

The hydrogen-ion concentration still appears to have some influence on the stability of some Fluidextracts of Ergot and of the pure crystals of ergotamine tartrate. A p_H of around 3.00 appears to be the critical point where there is the least

deterioration. For some Fluidextracts of Ergot deterioration takes place regardless of the hydrogen-ion concentration.

The data in this report show *no definite conclusions that the deterioration of Fluidextracts of Ergot or a solution of the pure ergotamine tartrate is prevented by a definite hydrogen-ion concentration.*

The object of our present and future researches on this problem is to determine first the stability factors of a solution of the pure crystals of the alkaloid, and second, to apply these findings to the Fluidextract of Ergot.

CONCLUSIONS.

1. The Epinephrine-Reversal Method (Broom and Clark) is a reliable method for the biological assay of ergot.

2. The Chemical Method (Smith's) shows promising results and deserves further consideration.

3. The hydrogen-ion concentration appears to have some influence on the stability of the ergot alkaloids.

4. From the above data no definite conclusions can be formulated that a certain hydrogen-ion concentration prevents the deterioration of the fluidextract or a solution of the pure ergot alkaloid.

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ADDENDUM BY E. E. SWANSON.

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While this paper was being written and completed, more data on the problem of stability became available.

Aging experiments of samples of Fluidextract of Ergot containing 2% to 2.5% of buffer salts and reducing and oxidizing agents show some interesting results.

Fluidextract, with sodium dihydrogen phosphate, and reducing agents, sodium dihydrogen hypophosphite and sodium hydrosulphite, show questionable results; whereas, fluidextracts with oxidizing agents, potassium chlorate, potassium dichromate and potassium permanganate, show complete loss of potency after six months' aging.

The hydrogen-ion concentration factor in these experiments, together with a more complete report of the above data, will be discussed later in another article.

EXPLANATION OF ILLUSTRATIONS.

Figure I represents an isolated rabbit uterus (whole uterus) the upper curve one horn and the lower curve the other horn of the same uterus. Each strip is immersed in 100-cc. cylinders. Epinephrine injections were given every twenty minutes. The first injection of 0.5 cc. (1–200,000) ergotamine tartrate in the 100-cc. cylinder represents a dilution of 1–40,000,000 of ergotamine tartrate. This dose produced an inhibition of 16% for the upper strip and 62% for the lower strip. As shown in the figure, the inhibiting effects of the alkaloid disappeared in 20 to 40 minutes. The second injection of ergotamine 0.5 (1–200,000) or 1–40,000,000 dilution plus the same dilution of histamine produced an inhibition of 17% for the upper strip and 58% for the lower strip. Apparently, the same concentration of histamine as ergotamine did not antagonize or aid the inhibitory effects of ergotamine. The third injection to the upper strip of ergotamine, same dilution as the previous injections, with the same dose of tyramine, showed no change in the action of ergotamine. The lower strip with ergotamine and choline (1–100) or 1–10,000 dilution, did not show the same inhibitory effect. Apparently, choline 2000 times the concentration of ergotamine,

prevents the inhibitory effect of ergotamine. The last injection of ergotamine produced an inhibition of 20% for the upper strip and 50% for the lower strip. Thus doses of histamine, tyramine and choline equal to that of ergotamine, appear not to aid or antagonize the inhibitory action of ergotamine.

Figure II represents an isolated rabbit uterus (whole uterus) the upper tracing one horn and the lower tracing the other horn. Each strip is immersed in 100-cc. cylinders. Epinephrine injections were given every twenty minutes. The first inhibition represents ergotamine tartrate 0.5 cc. (1–200,000) or 1–40,000,000 dilution. The second inhibition was a mixture of equal parts of ergotamine tartrate and histamine phosphate. The third inhibition was ergotamine and tyramine equal parts to upper tracing, and ergotamine and acetyl-choline equal parts to lower tracing. The last inhibition represents ergotamine alone.

Thus apparently equal parts of histamine, tyramine and acetyl-choline with ergotamine do not prevent or aid the inhibitory effects of ergotamine.

Figure III represents a whole isolated uterus of a rabbit. The upper and lower tracings are the two horns or fallopian tubes. These strips were immersed in 100-cc. cylinders containing Locke-Ringer solution. The first inhibition of 18% and 16% was due to ergotamine tartrate 0.5 cc. (1–200,000) or 1–40,000,000. The second inhibition of 16% and 19% was obtained by the injection of solution "M" which is a mixture of one part of ergotamine and two parts of histamine, tyramine and acetyl-choline. The remaining figures are alternating doses of ergotamine alone and a mixture of ergotamine and the three amines.

Thus the amines in a concentration twice that of ergotamine apparently does not influence the action of ergotamine.

Figure IV represents a whole isolated rabbit uterus. The upper and lower tracing are the two horns or fallopian tubes. This uterus after each dose of alkaloid returned to normal or responded to epinephrine stimulation partially in 20 minutes and completely in 40 minutes. The doses marked "T" represent Ergotamine Tartrate Tablets (Sandoz). The inhibition of these tablets was 60% and 58% for the upper tracing and 45% and 64% for the lower tracing, or an average inhibition of 56.75%. The doses marked "A" represent Ergotamine Tartrate Crystals (standard solution). The inhibition of this standard solution "A" was 67% and 67% for the upper tracing and 67% and 77% for the lower tracing, or an average of 69.5%.

Thus the tablets (Ergotamine Tartrate No. 62128 Sandoz) assay 81.65% of the standard crystals of ergotamine tartrate.

Figure V represents a whole uterus, one horn only. F. E. Ergot U. S. P. (*A*) is the control. F. E. Ergot U. S. P. (*B*) is the same as F. E. Ergot (*A*) except that a current of air was passed through for 48 hours. The average inhibition for "A" was 35⅓% and the average inhibition for "B" was 33% or "B" assayed 93% of "A" or a loss of only 7% by 48 hours of aeration.

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A PHARMACOLOGICAL NOTE ON CIMICIFUGA.

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INTRODUCTORY.

Cimicifuga, popularly known as Black Cohosh, Black Snakeroot, Rattleroot, Rattlesnake Root, Bugbane, Bugwort, Squaw Root and Macrotys, is the dried rhizome and root of *Cimicifuga racemosa*, of the family of the *Ranunculaceæ*. This drug has enjoyed considerable vogue in the United States, and it was official in the British Pharmacopœia but dismissed from it in 1914. A number of its fluid preparations are still recognized in America and the resin obtained from cimicifuga, known as cimicifugin, or macrotin, can also be found on the market. In addition to the resin, which can be obtained in impure form by precipitating a saturated tincture of the root with water, cimicifuga contains a volatile oil and a bitter neutral substance. This drug was introduced into medical practice a hundred years ago by Young (1). In addition to Young's paper on cimicifuga, there is another early reference to this drug by N. S. Davies (2).

Cimicifuga preparations have been recommended for a number of different conditions. Sir Lauder Brunton recommends it as a stomachic, cardiac tonic and expectorant (3). The eighteenth edition of Hare's "Practical Therapeutics," published in 1922, cites as indications for its use the following clinical conditions: rheumatism, chronic bronchitis, chorea, amenorrhea and subinvolution and tenderness of the womb (4).

Even the earlier editions of Osler's "Principles and Practice of Medicine" recommended the use of cimicifuga in St. Vitus' dance, or chorea (5). In spite of these various indications to be found in books on therapeutics, there is no scientific experimental work on record concerning the pharmacology of Snakeroot. In connection with a study of various drugs, old and new, purported to exert an effect on uterine muscle, the present author has made an experimental investigation of the fluidextract of cimicifuga and of cimicifugin; and the results obtained are briefly recorded in this place.