SCIENTIFIC SECTION

THE ASSAY AND DETERIORATION OF ERGOT PREPARATIONS.

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The knowledge that ergot and its extracts deteriorate more or less rapidly is not new although it has only comparatively recently been clearly established by the application of newer and more accurate methods of assay. A great many of the studies on the deterioration of ergot have been made on specimens prepared in the laboratory. Thompson (1) and others have suggested that Fluidextracts of Ergot prepared on a small scale in the laboratory may differ in potency and stability from lots prepared on a manufacturing scale. The work here reported represents a two years' study of manufacturing methods, assay methods and standards as applied to ergot extracts made on a manufacturing scale.

As a result of these studies we have concluded that either of the assay methods used in this work is superior to the present Official Cockscomb Method for control of Fluidextract of Ergot. It is also our conclusion that no method of manufacture at present available will yield a Fluidextract of Ergot which is permanently stable. Since it is obvious that frequently opened and partially filled specimens of Fluidextract of Ergot deteriorate much more rapidly than unopened, completely filled bottles, it is important that Fluidextract of Ergot should pass from the manufacturer to the patient in an unopened package. With the possible exception of that intended for use in the large hospitals, Fluidextract of Ergot should be packaged only in one-ounce containers.

EXPERIMENTAL.

Methods of Assay.—The Cockscomb Method official in the U. S. P. X is unsuitable for studying changes in ergot and its extracts. Consequently the Rabbit Uterus Method of Broom and Clark (2) and the Chemical Colorimetric Method of Smith (3) have been used in this work. Of the various modifications which have been suggested for the Rabbit Uterus Method as originally described by Broom and Clark, we have used that described by Pattee and Nelson (4).

The Chemical Colorimetric Method as described by Smith has been slightly modified as follows: The removal of alcohol from the Fluidextract has been omitted since the alcohol does not interfere with the extraction and is of value in aiding the separation of emulsions. The ether solution of the alkaloids is not made up to volume and divided into aliquots as described by Smith, but, on the contrary, the entire ether extract from 5 cc. of Fluidextract is extracted with 1% tartaric acid. During the addition of the paradimethylaminobenzaldehyde reagent heating has been avoided by immersing the tubes in cold water. For the development of color we have used a Hanovia Mercury Lamp No. AC 154 at a distance of 18 cm. Parallel assays made using these modifications and by the exact technic as described by Smith have yielded identical results. Since sunlight and the lamp used as sources of light have yielded identical results, we have adopted the lamp due to its greater dependability.

Crystalline ergotamine tartrate has been used as a standard and all results are reported in mg. equivalent of ergotamine tartrate per cc. of fluids or per Gm. of solids. The standard has been dissolved in 1% tartaric acid solution to make a concentration of 1 mg. per cc., filled into ampuls and kept in a refrigerator. The standard has been freshly prepared every three months and has been checked against a similar solution prepared by Dr. M. I. Smith.

Deterioration of Fluidextract of Ergot.—Thirteen lots of Fluidextract of Ergot prepared on a manufacturing scale by the U. S. P. X process were assayed at three-month intervals over a period of one year. The results are summarized in Table I.

TABLE I.—CHANGES IN FLUIDEXTRACTS OF ERGOT AS RECORDED BY THE COLORIMETRIC AND BROOM-CLARK METHODS OF ASSAY.*

Fluidextract No.	Initial Assay.		3 Months.		6 Months.		9 Months.		12 Months.	
	Color.	B-C.	Color.	B-C.	Color.	B-C.	Color.	B-C.	Color.	B-C.
82158	0.60	0.60	0.41		0.32	0.20	0.15		0.10	0.10
82528	0.63	0.60	0.63		0.50	0.50	0.33		0.14	0.13
Do. unopened	0.63	0.60							0.61	0.50
82799	0.66	0.60			0.58		0.27		0.14	0.15
Do. unopened	0.66	0.60							0.39	0.35
83173	0.69	0.65	0.59		0.57		0.33		0.22	0.15
Do. unopened	0.69	0.65							0.38	0.35
83504	0.73	0.70	0.52		0.47		0.34		0.21	0.10
83772	0.63		0.63		0.44		0.29		0.12	
Do. unopened	0.63								0.49	
83857	1.00	1.00	0.86		0.69	0.65	0.42		0.25	
Do. unopened	1.00	1.00							0.85	0.70
84052	0.87	1.00	0.89		0.64	0.50	0.47		0.24	0.15
Do. unopened	0.87	1.00							0.67	0.40
84165	1.60		1.29		1.18	1.00	0.72		0.38	0.33
Do. unopened	1.60								1.05	0.90
84166	1.55	1.20	1.28		1.22	0.75	0.65		0.32	0.30
Do. unopened	1.55	1.20							1.00	0.95
84247	0.80	0.87	0.59		0.58	0.33	0.42		0.21	0.16
84248	1.17	1.25	0.97		0.90	0.70	0.61		0.27	0.25
84312	0.82		0.77		0.75		0.63		0.34	

* Amber bottles were completely filled after the initial assays and aliquots were removed at 3-month intervals for assay. In some cases parallel assays were made at the end of one year on unopened specimens. All results are reported in mg. equivalent of ergotamine tartrate per cc.

Those samples which were opened at three-month intervals for removal of aliquots for assay show at the end of one year an average deterioration of 75% by the Colorimetric Method and 80% by the Broom-Clark Method. The unopened samples at the end of one year show an average deterioration of 27% by the Colorimetric Method and 36% by the Broom-Clark Method. This demonstrates very clearly the harmful effect of only occasionally opening and removing part of the contents from a bottle of Fluidextract of Ergot. The individual variations in stability are very great. For example, of the eight unopened samples assayed at the end of one year by the Colorimetric Method, the most stable had lost only 3% and the least stable had lost 45% of its potency.

The agreement between the Colorimetric and Broom-Clark Methods is good in the case of newly manufactured fluidextracts. Of the ten fluidextracts for which

initial assays were made by both methods, only two (84052 and 84166) show a discrepancy of greater than 10%. The one showing the greatest discrepancy (84166) is one in which the initial color assay is very high.

Although both methods indicate the rate of deterioration, a poorer agreement between the two methods is observed in the older fluidextracts which have undergone deterioration. In almost every case where the two methods show a disagreement, the Broom-Clark Method has yielded lower results than the Colorimetric Method. Thus a preparation which tests low by the Colorimetric Method has, in our experience, invariably tested low by the Broom-Clark Method, but the reverse is not necessarily true. Since the biologically inactive alkaloids of ergot are active in producing color, our results would indicate that as Fluidextract of Ergot deteriorates the disappearance of active alkaloids is likely to be greater than that of the inactive alkaloids. We have no evidence, however, that the biologically active alkaloids are converted into inactive alkaloids during the process of deterioration. That such a conversion can occur is indicated by an experiment of Wokes and Crocker (5) in which an 18 months old ergotoxine phosphate solution was found by Colorimetric assay to have 66% of its original activity and by Broom-Clark assay only 10% of its original potency. Such a change as this can only be explained on the basis of a conversion of active into inactive alkaloids.

Wokes and Crocker (5) have severely criticized the Colorimetric Method of Smith because it measures the inactive as well as the active alkaloids. However, the biologically inactive alkaloidal content of Fluidextract of Ergot prepared on a manufacturing scale is seldom high enough to interfere significantly with the Colorimetric Method in measuring biological potency. Consequently the method is of great value in controlling the manufacturing process. But in view of the fact that occasionally a lot of ergot is encountered which is high in the inactive alkaloids it is well to check the potency of the finished extract by the Broom-Clark Method.

TABLE II.—CHANGES IN FLUIDEXTRACTS OF ERGOT AS AFFECTED BY ADJUSTMENT OF p_H.*

Fluidextract No.	Initial. Color.	Initial. B-C.	3 Months. Color.	3 Months. B-C.	6 Months. Color.	6 Months. B-C.	9 Months. Color.	9 Months. B-C.	12 Months. Color.	12 Months. B-C.
81889										
Natural p_H 4.7	0.51	0.38	0.44		0.35	0.25	0.29		0.24	0.20
Do. adjusted p_H 3.0	0.51	0.38	0.39		0.29	0.15	0.19		0.13	0.10
Do. adjusted p_H 6.1	0.51	0.38	0.39		0.29	0.08	0.09		0.07	0.05
82712										
Natural p_H 4 6	0.61	0.65	0.60		0.55		0.54		0.45	0.33
Do. adjusted p_H 3.0	0.61	0.65	0.55		0.54		0.49		0.41	0.22
Do. adjusted p_H 6.1	0.61	0.65	0.48		0.43		0.38		0.28	0.15
87380										
Natural p_H 4.6	0.81				0.57					
Do. adjusted p_H 3.0	0.81				0.36					

* The conditions of storage and assays are the same as for the fluidextracts reported in Table I. Results reported in mg. equivalent of ergotamine tartrate per cc.

Effect of p_H Adjustment upon the Stability of Fluidextract of Ergot.—Three lots of Fluidextract of Ergot prepared by the U. S. P. X process were found to have a p_H of 4.6–4.7. Aliquots were adjusted to a p_H of 3.0 by the addition of hydrochloric acid and to a p_H of 6.1 by the addition of sodium hydroxide. Assays made immediately after the p_H adjustment show that no loss of alkaloidal potency occurred during the addition of acid and alkali. However, later assays, summarized in Table II, indicate that this adjustment of p_H had an unfavorable effect upon the stability of the extracts. The samples with a p_H of 3.0 are in every case less stable than the unadjusted sample and the two samples adjusted to a p_H of 6.1 are the least stable of all.

In his earlier work Swanson (6) concluded that a p_H of 3 or less is necessary for the stability of Fluidextract of Ergot. However, more recently, Swanson (7) *et al.*, somewhat modifies his former conclusions. Smith and Stohlman (8) conclude that variation of p_H from 5.2 to 2.2 does not favor the stability of Fluidextract of Ergot.

Our conclusions are that variation of p_H between the limits of 3.0 to 6.1 does not enhance the stability of Fluidextract of Ergot. Manipulation of p_H in the finished fluidextract appears to be more harmful than beneficial.

Changes in Fluidextracts of Ergot as Affected by Frequent Opening of the Bottle for Removal of Part of the Contents.—The results summarized in Tables I and II indicate the extremely rapid deterioration of samples which are opened at three-month intervals. The conditions under which this deterioration occurs are not essentially different from those under which Fluidextract of Ergot is dispensed. The physician or druggist who buys this preparation in pint or gallon containers is compelled to subject the contents to opening at intervals considerably more frequent than three months.

In order to study the effects of such manipulation two lots of Fluidextract of Ergot were set aside in large bottles. They were opened once every two weeks and one ounce of the contents removed. At the end of six months, assays on the remainder of the contents showed a loss of 76 and 74% of the original alkaloidal potencies. Control, unopened samples of the same lots assayed in parallel were found to have lost only 14 and 3%, respectively, of their original potencies.

These results confirm those reported above in showing that under conditions of frequent opening and partial removal of the contents, Fluidextract of Ergot may become practically inactive in only a few months. This rapid deterioration can be greatly reduced, although not completely eliminated if the manufacturer will package Fluidextract of Ergot only in one-ounce containers, which will reach the consumer in an unopened condition.

Attempts to Stabilize Fluidextract of Ergot by Bottling under Different Gases.—Since Fluidextract of Ergot deteriorates rapidly when frequently exposed to the air, the question arose as to whether a stabilization could be accomplished by excluding air at the time of bottling. Samples from a single lot of fluidextract were bottled in one-ounce amber bottles under air, nitrogen, carbon dioxide and oxygen. The oxygen samples were treated with a vigorous stream of oxygen for two hours before being stoppered. This resulted in only a slight immediate loss of potency as measured by the Colorimetric Method but promoted a very rapid subsequent deterioration. The CO_2 samples were saturated with this gas before being

stoppered. In this entire series a new, unopened bottle was taken for each assay, and the relative stabilities of the groups are summarized in Table III.

TABLE III.—CHANGES IN FLUIDEXTRACT OF ERGOT AS AFFECTED BY BOTTLING UNDER DIFFERENT GASES.*

Fluidextract.	Initial. Color.	B-C.	3 Months. Color.	B-C.	6 Months. Color.	B.-C.	9 Months. Color.	B-C.	12 Months. Color.	B-C.
Under air	1.30	1.00	1.05		0.85	0.65	0.73		0.70	0.55
Under N_2	1.30	1.00	0.99		0.92	0.75	0.65		0.18	0.20
Under CO_2	1.30	1.00	1.10		0.78	0.60	0.77		0.71	0.60
Under O_2	1.20	1.00	0.47		0.33	0.20	0.24		0.21	0.15

* All assays made on previously unopened specimens. Results reported in mg. equivalent of ergotamine tartrate per cc.

As would be expected, the oxygenated fluidextract proved to be the most unstable of the series. The fluidextracts bottled under nitrogen and carbon dioxide are no more stable than those bottled under air in the usual way. We are unable to account for the rapid deterioration of the nitrogen sample during the last three months of the test, This sample was assayed at twelve months on three different bottles with identical results. Consequently it is not a condition peculiar to a single bottle of the nitrogen series.

Although oxidation is probably an important factor in the deterioration of ergot alkaloids, our attempts at stabilization of the fluidextract by the exclusion of air and bottling under an inert gas have been unsuccessful. Similarly the attempts of Rowe and Scoville (9), Smith and Stohlman (8), and Powell, Schulze and Swanson (10) to stabilize Fluidextracts of Ergot by the use of reducing agents and buffers have yielded irregular and inconclusive results.

The Deterioration of Amine-Free Fluidextracts of Ergot.—Thompson (11) has developed a process for manufacturing a Fluidextract of Ergot in which the amines are removed by a preliminary extraction with weak alkali. The chief advantage of such an extract over the regular U. S. P. product appears to be that removal of the interfering amines eliminates one of the many sources of error in standardizing the Fluidextract by the Cockscomb Method.

In order to determine whether the removal of amines increases the stability of Fluidextract of Ergot, four lots were prepared by Thompson's method and studied over a period of one year. By both the Colorimetric and Broom-Clark Methods of assay these were found to be no more stable than the regular U. S. P. Fluidextracts reported in Table I. Consequently we conclude that the removal of amines does not lessen the deterioration of Fluidextract of Ergot.

The Stability of Powdered Extracts of Ergot.—Powdered extracts of ergot have been prepared on a manufacturing scale by concentrating the fluidextract to dryness *in vacuo*. This process requires prolonged heating which is known to have an adverse effect upon the stability of Fluidextract of Ergot. By such a process the yield has been found to be from 70 to 90% of the alkaloidal potency of the fluidextract used as starting material. Three lots of powdered extract of ergot prepared in this way have maintained their potency without detectable loss over a period of one year. These samples were stoppered in amber and flint bottles and assayed by both the Colorimetric and Broom-Clark Methods. Our findings are in agreement with those of Oettel (12) who reports that dry extracts of ergot show

no loss of potency during a period of eight months in contrast to a loss of two-thirds of the potency occurring during the same period in the fluidextracts from which the dry extracts were prepared. Thus, we conclude that the dried extract of ergot is the most stable crude alkaloidal preparation thus far obtained and stands in marked contrast to the instability of crude ergot and its fluidextract.

Consideration of Standards.—The standard Fluidextract of Ergot which has been official in the U. S. P. X is unsatisfactory because of its instability. Although ampuled *in vacuo*, the most recent official standard No. 2160 is no more stable than many market samples of Fluidextract of Ergot. Our assays have shown that the standard Fluidextract No. 2160 has shown a progressive loss of potency amounting to 43% by the Broom-Clark Method and 37% by the Colorimetric Method during a period of 20 months.

Crystalline ergotamine tartrate and ergotoxine ethanesulphonate have both been suggested as ergot standards. Either of these is apparently stable in the dry form if protected from air, moisture and light, and offers a marked improvement over the present fluidextract standard. Solutions of these salts in 1% tartaric acid when ampuled and kept in a refrigerator are stable for at least six months. As a result of numerous assays we conclude that ergotamine tartrate (Sandoz) and ergotoxine ethanesulphonate (Burroughs, Wellcome and Company) are equivalent by the Colorimetric Method, but by the Broom-Clark Method one mg. of ergotoxine ethanesulphonate is equal to 1.25 mg. of ergotamine tartrate.

Consequently, the use of the ergotoxine salt as a standard for assaying Fluidextract of Ergot will tend to cause a 25% disagreement between the Broom-Clark and Colorimetric Methods of assay.

SUMMARY.

The more or less rapid and variable deterioration of Fluidextract of Ergot has been confirmed. Since frequent opening and exposure to air is inducive to an extremely rapid deterioration, it is recommended that Fluidextract of Ergot be put up in small unit packages to insure transfer to the patient in the original, unopened container. Adjustment to a p_H of 3.0 or removal of amines does not increase the stability of the fluidextract. The powdered extract appears to be the most stable crude ergot preparation.

The chemical Colorimetric Method of Smith gave results agreeing sufficiently closely with the Broom-Clark Biological Method to make it extremely valuable for controlling the manufacture of Fluidextract of Ergot. Either of these methods is superior to the Cockscomb Method. Either crystalline ergotamine tartrate or ergotoxine ethanesulphonate will make a much more satisfactory standard than the present unstable standard fluidextract.

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STUDIES IN THE EXTRACTION AND HYDROGEN-ION CONCENTRATION OF TINCTURE OF DIGITALIS.

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INTRODUCTION.

In a previous communication to THIS JOURNAL the authors (1) studied the relationship between the potency and the hydrogen-ion concentration of tincture of digitalis. In this investigation it was shown that the tincture prepared with dehydrated alcohol as a menstruum, differed from that tincture prepared with 80 per cent in at least three respects. *First*, the tincture prepared with dehydrated alcohol showed a greater hydrogen-ion concentration, *second*, a comparatively negligible amount of ash was present and *third*, the heart tonic value was markedly less than that exhibited by the tincture prepared from the same drug with 80 per cent alcohol.

Since the publication of this work, the comprehensive investigations of Hoekstra (2) have come to the authors' attention. Among other interesting observations, this investigator showed that a tincture prepared with 50 per cent alcohol contained 3.8 cat units of digitoxin, 2.5 cat units of gitalin and 7.3 cat units of bigitalin. Whereas a tincture prepared from the same powder using dehydrated alcohol as a menstruum contained $^3/_8$ the digitoxin content, $^5/_8$ the total bigitalin content and only a trace of gitalin. Wokes (3), after a study of the potency and total solids of tincture of digitalis, concluded that no definite relationship could be established between these factors. Of striking significance is the observation of Stasiak and Zboray (4) who found less residues from the tinctures made with dehydrated alcohol than those prepared with diluted alcohol. In addition, the tincture prepared with dehydrated alcohol showed the lowest potency when assayed by the cat method, but by the frog method the tinctures prepared with dehydrated 70 per cent and 50 per cent alcohol, showed essentially the same potency.

In this study the authors have extended their investigations mentioned in a foregoing paragraph and attempted to correlate their findings with the work of other workers.

EXPERIMENTAL.

Through the courtesy of the Upsher Smith Company and Penick Company, six authenticated samples of digitalis (*purpurea*) were obtained. From these, six tinctures were prepared by the official method. The removal of fat was omitted. Six tinctures were prepared using dehydrated alcohol as a menstruum and from the marcs obtained in this extraction six other tinctures were prepared using 80 per cent alcohol as a menstruum.