FURTHER STUDIES CONCERNING THE U. S. P. X BIO-ASSAY STANDARD FOR ERGOT AND FLUIDEXTRACT OF ERGOT.*

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INTRODUCTION.

The bio-assay requirements of the U. S. P., 10th Revision, for Ergot and Fluidextract of Ergot, involves the use of a "Standard Fluidextract of Ergot" as a standard for comparison of potency.

That a standard for comparison, in connection with the assay of ergot or its fluidextract, will be necessarily included in the 11th Revision of the U. S. P., is a foregone conclusion. In an earlier report of experimental studies on Ergot, the author (1) recommended that the Epinephrine-reversal Rabbit Uterus Method of Broom and Clark be adopted by the next Pharmacopœia in place of the now official Cock's Comb Method because of the greater accuracy and dependability afforded by the former. At approximately the same time, Smith (2) reported a colorimetric method which shows much promise for evaluating the specific alkaloidal content of ergot or its preparations. It is not the purpose of this report to discuss, or offer experimental data relating to methods. It is desired only to point out that there are at present but the three above-mentioned methods to be considered as possibilities by the U. S. P. Committee of Revision, and that each of these three methods, whether biological or colorimetric, necessitates the use of some standard of comparison. Further studies on methods will appear at a later date.

The method of preparation and nature of the current bio-assay standard for Ergot and Fluidextract of Ergot are specifically described in the U. S. P., (3) and, therefore, will not be repeated here. It is desired to point out, however, that in providing for this standard fluidextract, absolute stability was apparently assumed, since no provision was made for preparing successive lots which would be necessitated by possible deterioration. In a previous report (4) dealing with the adaptability of the official standard fluidextract for its intended purpose, experimental evidence was presented to show that the official standard was not absolutely stable, the experimentation being carried out upon preparations prepared and stored exactly as is the standard fluidextract, although direct observations had not been carried out upon the actual standard. For the purpose of determining whether or not such deterioration actually did occur in the standard fluidextract, the stability of all lots of this preparation distributed by the U. S. Department of Agriculture has been investigated, this data constituting the basis of this report.

Since the U. S. P. X became official, three different lots of the standard fluidextract have been prepared and distributed by the U. S. Department of Agriculture. The first, which was distributed from 1926 to 1928, was designated as "P.C. 635." When this lot was practically exhausted, a succeeding lot was pressed into use, and designated as "P.C. 636." This second lot constituted the official standard until February 1930. On approximately the latter date, lot "P.C. 636" was practically exhausted, and a third lot, designated as "P.C. 2160" was pressed into use and at the date of this writing, is still distributed as the official standard.

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In an earlier communication (4) it was pointed out that an ideal standard for the bio-assay of crude ergot, or its preparations, must meet two fundamental requirements. *First*, its pharmacodynamic activity must be representative of the clinically desirable activity of ergot; and, *second*, it must be constant in activity and composition. The author (4), as well as a number of other investigators, (5), (6), etc., have experimentally shown that the official standard fluidextract fully meets the first of the two fundamental requirements. On the other hand, the earlier work of the writer (4) strongly indicated that the second requirement, *i. e.*, that of stability and constant composition, was not adequately met by the present standard. Because of this apparent lack of stability in the official standard and the fact that ergotoxine ethanesulphonate and ergotamine tartrate proved to be distinctly superior to the official standard regarding constancy of composition and stability, as well as meeting the first requirement, it was recommended (1) that one of two alternatives be adopted in the next Pharmacopœia as follows: *First*, the use of one of the above-mentioned crystalline alkaloidal salts as the standard; or, *second*, the use of the U. S. P. X Standard Fluidextract, which had been accurately adjusted to a definite potency by comparison with one of the crystalline alkaloidal salts, and replacing each lot as soon as perceptible deterioration became evident through frequent check assays by comparison with the salt of ergotamine or ergotoxine.

EXPERIMENTAL.

To determine conclusively whether the U. S. P. X Standard did or did not lose potency with age, observations have been made directly upon all three lots which have been used, the potency being determined by comparison with the same lot of ergotamine tartrate employed as a standard in the earlier work. Since, in the experience of the author, the Epinephrine-Reversal Method of Broom and Clark has proven to be the most accurate and dependable of all methods yet proposed, this has been the method employed in this work. Because the greatest possible accuracy, well within 10%, was necessary, the greatest of care was exercised to select rabbit uteri of maximum sensitivity, and many determinations were carried out upon each lot of the Standard at various intervals.

Although U. S. P. X mentions nothing regarding the p_H of the Standard Fluidextract, it is now known that this factor plays a major part in the stability of any Fluidextract of Ergot. Accordingly, p_H determinations have been included in the study. For the purpose of conserving the relatively small amounts of the respective lots of the Standard Fluidextract available, the following set-up, using the electrode vessels recommended by Cullen and Biilmann for small quantities of solution, was employed:

Pt	:	KCl 0.09 M.	:	KCl	:	Ergot solution	:	Pt
	:	HCl 0.01 M.	:	Sat'd.	:		:	
	:	Quinhydrone	:		:	Quinhydrone	:	

The system was tested and checked very thoroughly before proceeding, using a standard HCl solution, the p_H of which was accurately known.

The results obtained, including the p_H of the three lots of the U. S. P. X Standard Fluidextract, and the potency in terms of Ergotamine base and Ergotamine

Tartrate at the beginning and end of an eighteen-month interval, are tabulated as follows:

Std. F. E. lot no.	Present age (approx.)	Potency, Feb. 1930 in Terms of Ergotamine tart.	Potency, Feb. 1930 in Terms of Ergotamine base.	After 18-Mo. Interval, Potency July 1931 in Terms of Ergotamine tart.	After 18-Mo. Interval, Potency July 1931 in Terms of Ergotamine base.	Per cent deterioration in 18 months.	p_H.
PC 635	5 years	0.235 mg./cc.	0.198 mg./cc.	0.210 mg./cc.	0.177 mg./cc.	10.6%	4.192
PC 636	3 years	0.290 mg./cc.	0.245 mg./cc.	0.275 mg./cc.	0.232 mg./cc.	5.7%	1.778
PC 2160	18 mos.	0.600 mg./cc.	0.507 mg./cc.	0.549 mg./cc.	0.464 mg./cc.	8.4%	1.809

DISCUSSION.

From the above experimental data, it becomes readily apparent that no lot of the U. S. P. Standard Fluidextract is absolutely stable. On the other hand, it is equally evident that this standard, during a period of eighteen months, deteriorates to so slight an extent as to be practically impossible of significant detection by the less accurate official Cock's Comb Method. By the Rabbit Uterus Method, every determination upon each lot clearly indicated significant, though slight, loss of potency, the tabulated results in every instance being the average of numerous determinations. Replacing each lot of the standard *after one year of service*, by another lot accurately adjusted to a definite potency in terms of ergotamine tartrate or ergotoxine ethanesulphonate, is apparently the only way by which a practically constant potency in the present standard could be insured.

The relationship of p_H to deterioration in the different lots presents an interesting subject. Lot "P.C. 635," whose p_H value is highest, shows, as might be expected, the greatest deterioration. "P.C. 636," however, whose p_H value was even slightly lower than "P.C. 2160," deteriorated in potency to possibly a lesser degree than did "P.C. 2160." It is true that the difference is very slight, but the difference was so consistent as to prove rather convincing. If the difference is actual, it is probably best explained by the hypothesis that "P.C. 636" aged longer than "P.C. 2160," and had attained a slightly greater degree of stability.

SUMMARY.

The stability of all lots of the U. S. P. X Standard Fluidextract of Ergot used up to the present time has been experimentally determined during a period of eighteen months. The p_H values of these three lots have likewise been determined and included in the results.

CONCLUSIONS.

1. The U. S. P. X Standard Fluidextract is shown to deteriorate to such an extent during eighteen months as to become perceptible to the sensitivity of the Isolated Rabbit Uterus Method of assay, although the deterioration during this period is less than could be significantly detected by the less accurate official Cock's Comb Method.

2. To insure a practically constant potency in the official standard, a new lot should be pressed into use at the end of every twelve months.

3. Either of the dry alkaloidal salts, Ergotamine Tartrate or Ergotoxine Ethanesulphonate, properly prepared and handled, would constitute a standard superior to the one at present official. Solutions should be freshly prepared for use.

My thanks are due to Mr. C. T. Ichniowski, of this Department, and to Prof. E. G. Vanden Bosche of the Department of Physical Chemistry, for the p_H determinations included in these studies.

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(4) M. R. Thompson, JOUR. A. PH. A., 19 (1930), 446.
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FLUIDEXTRACT OF ERGOT.*

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The physiological activity of Ergot having been established as being mainly due to alkaloids which are insoluble in water and soluble in alcohol, and these alkaloids having been shown to be instable, being easily oxidized, it seems reasonable to judge that more stable preparations of Ergot may result from the use of more strongly alcoholic menstrua and the use of reducing agents. This paper is designed to throw light on the subject from this angle.

The large amount of chemical and pharmacological work that has been done on ergot during recent years has disclosed three facts of pharmaceutical interest. We now believe that the physiological action of ergot is due almost entirely to its alkaloids; that these alkaloids, when free, are soluble in alcohol but almost insoluble in water, and that the alkaloids are instable, probably becoming oxidized easily and thus losing their potency. These facts help to suggest some ways of producing more stable preparations of ergot.

The following experiments were made with this end in view, the fluidextract being selected for study.

A quantity of ground and defatted Spanish ergot sufficient for all the preparations made was used, the results thus being comparative and the method of treatment being as nearly the same as was practicable in all cases.

The fluidextracts were all made by the general process directed for Fluidextract of Ergot in the Pharmacopœia, namely, percolation of the drug, concentration of the weaker portion of percolate by distillation under reduced pressure and solution of the concentrate in the reserved portion. Heat was thus used in making all samples. The fluidextracts varied in the alcoholic strengths of the menstruum used, the amount and character of the acid employed and in a preliminary sterilization of the drug, in some cases. Ergot being the result of a fungus blight may well be expected to contain several enzymes, perhaps among them an oxydase or peroxydase and the effect of sterilizing these is a part of the attempt to secure stability. The sterilizing was accomplished by moistening the drug thoroughly with 95 per cent (or in two cases with 77 per cent) alcohol and heating the mixture under a reflux condenser, the hot alcohol thus acting as the sterilizing agent. The alcohol so used afterward became a part of the menstruum for extraction, thus avoiding drying and loss.

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