SCIENTIFIC SECTION

THE EXTRACTION AND ASSAY OF CRUDE ERGOT.[1]

(From the Department of Biochemistry, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

BY MARVIN R. THOMPSON.[2]

A report of studies embracing the efficiency of various methods of extraction of crude ergot and the evaluation of the alkaloidal activity by biological and chemical methods, including potentiometric studies. The isolation of a new active substance from Ergot, with pharmacologic and chemical studies thereof, likewise constitutes a part of this report.

The United States Pharmacopœia, 10th Revision, requires Ergot to be converted into a fluidextract, by the official process for preparing Fluidextract of Ergot, before assay. This official process, aside from the menstrua employed, is one of the general processes prescribed for Fluidextracts in the U. S. P., designated as Type Process "B." (See U. S. P. X, for details of process.)

The importance of efficiency in extraction by this process cannot be overestimated for two major reasons:

1. Because if this process does not result in the appearance of the total amount of therapeutically active principles that was present in the parent drug, no method of assay, regardless of how accurate it may be, could yield reliable results in evaluating the crude drug.

2. Because of the economic factor in the manufacture of standardized Fluidextract of Ergot. Incomplete extraction would necessarily result in a lower yield of finished product of standard potency.

In an earlier series of reports, the author (1) confirmed the work of several European workers in showing that the U. S. P. X process for preparing a Fluidextract of Ergot was far more efficient than some of the more important pharmacopœial processes, and second to none in extracting the alkaloids which are at present generally regarded as therapeutically valuable constituents of the drug. This, however, does not necessarily indicate that the present extraction process is perfectly satisfactory.

Wokes and Elphick (2) found that, working with 20-Gm. samples, the U. S. P. menstruum No. 1 for Fluidextract of Ergot did not exhaust the drug until the percolation was carried to at least a 1 in 6 percolate. It has, of course, been known for many years that a 1 to 1 percolate would not contain the total alkaloids of the drug. Accordingly, the U. S. P. process requires that a reserve portion of

NOTE: The work herein described concerning the extraction of Ergot constitutes the basis for a recommendation to Prof. E. Fullerton Cook and Dr. Erwin E. Nelson, of the Committee of Revision, during a visit to my laboratory in November 1931, to change the method of preparation of Fluidextract of Ergot, U. S. P. X, from Type Process "B" to Type Process "C" for Fluidextracts of the U. S. P. X, for the proposed Interim Revision on Ergot and Fluidextract of Ergot.

[1] Part II of a Thesis submitted to the Faculty of Philosophy of the Johns Hopkins University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Emerson Professor of Pharmacology, School of Pharmacy, University of Maryland.

850 cc. per 1000 Gm. of drug be obtained, then that percolation be continued until the drug is exhausted. No test for exhaustion is mentioned. The large volume of exhaust percolate is then directed to be concentrated, with the aid of heat, to a volume such that when incorporated in the reserve percolate, one cubic centimeter will be the equivalent of one Gm. of drug. The prescribed U. S. P. assay procedure assumes that this Fluidextract contains the total activity of the drug.

It is now well known that the prolonged heating necessary for concentration of the exhaust percolate, even *in vacuo*, is not conducive to the stability of Ergot alkaloids. The work of Wokes and Elphick (2), Smith and Stollman (3), and the writer (1), conclusively establishes this point.

This being the case, even if the assay method were satisfactory, it is highly probable that instead of obtaining an accurate estimate of the total alkaloidal potency of the drug sample itself, *one obtains an estimate of the activity of a Fluidextract prepared on a laboratory scale, yielded by the drug sample.* This Fluidextract would, because of the heat employed in concentrating the exhaust percolate, probably contain less alkaloidal potency than was present in the parent drug, and consequently a low assay result would be obtained.

Since a great portion of the total crude Ergot used in this country is used in preparing the official Fluidextract, this would not be a seriously objectionable situation, provided that the extraction efficiency experienced on a laboratory scale was the same as that found in the commercial manufacturing scale. As it happens, this is not the case.

All other factors, such as composition of menstruum, time of maceration and percolation rate, etc., being equal, the efficiency of any ordinary extraction procedure is dependent upon the fineness of the particles of the drug, and the height of the column of drug through which the menstruum passes. At the same time, it is probably true that the extraction equipment and procedure of no two manufacturers is of identical efficiency. But it is most certainly true that the results of an assay of crude Ergot (as the U. S. P. directs, using an assay sample of the usual size) are rarely confirmed by the results obtained in an assay of a finished Fluidextract prepared on a commercial scale.

The experimental evidence which follows bears upon the points raised above.

In order to secure reliable results in assaying strong and weak percolates, the method of assay employed will be described in detail, and reasons for rejecting other methods given.

Assay Methods Available.

1. The U. S. P. X Cockscomb Method and Modifications.

Owing to a number of factors, such as the rapid change in the susceptibility of so-called "standardized cockerels" from week to week, the presence of substances in ergot extracts which interfere with the quantitative response to the ergot alkaloids (a circulatory phenomenon), the very lack of uniform sensitivity of the cockscomb reaction to varied doses of ergot preparations, the hideously discrepant results obtained by different assayists upon identical samples, the fact that tests were desired on relatively low amounts of alkaloids in unusually great volumes

of percolate (which would necessitate impossible doses to each cockerel), this method was rejected as a possible assay procedure in this work.

Concerning the mechanism of this reaction, it is significant to note that any kind or intensity of the characteristic Ergot cyanosis (bluing) can be duplicated by simply exerting pressure, with the fingers, or a suitable weak clamp, around the base of the comb for ten to forty minutes. This indicates the reaction to be of vasomotor character, and that the quantitative response to Fluidextract of Ergot could consequently be expected to be just as erratic as that shown upon the carotid blood pressure of dogs or cats following subcutaneous, intramuscular or intravenous administration of this preparation.

2. THE COLORIMETRIC METHOD, AND MODIFICATIONS.

After many attempts, our inability to obtain satisfactory check results by this method as described by Smith (4) before this phase of the work was undertaken, caused the abandonment of this method for the purpose at hand. The discrepancies obtained were apparently due to lack of sufficient experience with the method, but since then, this method and its various steps and modifications have been studied in great detail. The results of these studies form a later part of this report.

3. THE ISOLATED RABBIT UTERUS METHOD.

An earlier series of articles by the writer (1) dealing with various phases of the Ergot problem was terminated by a series of recommendations, among which the so-called "Epinephrine-reversal" Rabbit Uterus Method, essentially that of Broom and Clark (5), was set forth as the most accurate and most reliable biological method then available for the estimation of the alkaloidal activity of Fluidextract of Ergot. No worker, before or since then, has published evidence which would cause this view to be altered. It is significant that those workers who have had reasonable experience with this method agree that it is more accurate and more reliable than the present official method.

In 1930, however, M. I. Smith (4) published his chemical method, and showed with an impressive amount of evidence that this method was as accurate and dependable as the Broom-Clark Rabbit Uterus Method, and decidedly less time-consuming. Studies on this method will be deferred to a later part of this report, since it was not successfully used on the phase of work at hand.

Since practically every worker of experience upon the Broom-Clark Method has agreed that this is the most accurate and reliable of all biological methods proposed, this view is now quite generally accepted. Nevertheless, there is a very considerable amount of opposition to the method, on the grounds that it is too difficult of technique, too tedious, too time-consuming, and, therefore, not practical as a routine method in laboratories having large numbers of these assays to perform.

It is important to briefly discuss specifically these objections to the method.

First of all, using the method essentially as originally described as has been customary by all workers to date, the very fact that the method involves the use of isolated segments of the rabbit uterus has in itself caused some apprehension.

Secondly, all writers have experienced considerable difficulty in selecting suitable uteri. Various views have been expressed on how best to choose the

rabbits, but no practical method has been found to insure the selection of anything but a fair proportion of satisfactory uteri. Pregnancy, age of rabbit, and stage of oestrous are the interfering factors.

Thirdly, and perhaps the greatest objection of all, the time required to run an assay compared to that required in the official Cockscomb test, has been far too great. It appears to be a quite general view that an operator must be exceedingly fortunate to obtain a satsifactory assay on more than one sample during a whole day.

The writer has devoted a great deal of time during the past two years in a continuation of previously reported work on Ergot. In the course of this work, the Rabbit Uterus Method has been depended upon almost exclusively for estimations of alkaloidal activity, and a technique has been developed which, at least partially, eliminates hitherto existing objections to this method.

First of all, this technique provides for accuracy and dependability in estimating the alkaloidal activity that cannot be duplicated by any other known biological method in our hands.

Secondly, this technique provides for an assay upon a sample of Fluidextract of Ergot, that is sufficiently accurate for all practical purposes, in a period of time not exceeding two hours.

Thirdly, the necessity for blaming poor results upon unsatisfactory uteri has been practically eliminated.

Lastly, the technique does not require expensive equipment or an unreasonable amount of experience, as has been proven to the writer's satisfaction by the fact that students have demonstrated their ability to secure dependable results after using the technique only a relatively short period of time.

In support of these claims, it is necessary to describe this technique in detail.

(To be continued)

DIGITALIS FAT—THE PETROLEUM-ETHER EXTRACTIVE OF DIGITALIS PURPUREA LINNÉ.*

BY A. JOHN SCHWARZ.

INTRODUCTION.

Reports on the pharmacological action of Digitalis and various preparations of the leaf make mention of the presence of a "fixed oil" or "fat" in the leaf which may or may not render the preparation (particularly the tincture)

a. Less sightly
b. Less stable
c. Less palatable
d. Less tolerable.

The last three conditions apply also to the leaf.

It is strange that no detailed chemical analysis of the digitalis fat has been recorded since the above-mentioned characteristics have been ascribed to it. Such

* An excerpt from the thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin, Sept. 1931, and presented before the Scientific Section of the A. PH. A., Toronto, Aug. 1932.