SCIENTIFIC SECTION

THE PHARMACOLOGY OF ERGOT: WITH PARTICULAR RESPECT TO ITS BIOLOGICAL ASSAY AND STANDARDIZATION.*

(The bibliography will follow the last article of the series.)

I. THE PRESENT STATUS OF ERGOT.

BY MARVIN R. THOMPSON.[1]

INTRODUCTION.

During the past fifty years a vast amount of experimental work has been reported upon the pharmacological and clinical properties of ergot of rye. As a drug it is comparatively well known, having been used in medicine for a great many years, principally as a parturient or parturifacient in the treatment of post-partum hemorrhage, because of its specificity in producing contraction of the uterus, especially in the gravid state.

In spite of the fact that the drug has been found to be highly specific in its physiological and clinical reactions, in recent years it has fallen into more or less disrepute as a medicinal agent, largely because of certain inconsistencies and irregularities arising from its use. Posterior pituitary substance has been found to possess similar clinical properties with a less uncertainty in connection with its therapy, and has, therefore, threatened to replace ergot, especially in obstetrics.

From the amount of ergot imported and used in this country alone in recent years, however, it is clearly evident that this drug still occupies an important place in medicine as a parturient and also as an emmenagogue. Since no substitutes have supplanted its use in therapeutics, it is obvious that the valuable and important properties of the drug still receive recognition.

Because of this evident value of ergot in medicine, and the highly specific nature of the drug as shown in clinical and pharmacological studies, the unsuccessful use of the drug in most instances is believed to be due to lack of knowledge concerning the preparations used, and the inconsistencies in reactions exhibited by its galenicals may be logically ascribed to one or more of the following factors: 1st, that individual samples of the crude drug are inconsistent in regard to the amount or proportion of active principles contained therein; 2nd, that the processes involved in the preparation of its galenicals are faulty in that they do not always uniformly extract the specific active principle or principles; 3rd, that the methods used for the standardization or adjustment of potency do not accurately measure the clinically active constituents; 4th, that one or more of the active principles may seriously interfere with the clinical effect of the others, or interfere in the measurement of potency by the currently used bio-assay methods; 5th, that the medicinally valuable principles are of such an unstable nature that they do not appear in the preparations in defined amount by the time they are administered to the patient due to deterioration of the active agents.

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* Scientific Section, A. PH. A., Rapid City meeting, 1929.

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In an attempt to determine which of these factors are responsible for the clinical and pharmacological inconsistencies or irregularities, the author has conducted an intensive investigation of the pharmacological properties of ergot of rye during the past two years. The results of these experiments, it is believed, explain many of the currently existing disagreements among clinicians and pharmacologists regarding the nature of its reactions.

In reviewing the earlier literature upon ergot, one encounters such a long series of conflicting results and differences of opinion that the subject is truly bewildering, particularly that dealing with the active principles found in the drug, and the chemical nature and pharmaco-dynamic activity ascribed thereto.

During the past 25 years, several investigators have greatly overcome the previously existing chaos by a re-interpretation of the earlier results of others, which was made possible only by intensive research. Barger and Dale (6), in 1907, published a remarkably enlightening interpretation of earlier literature upon the constituents of ergot together with their own observations, which, together with more recent work, quite definitely established that the specific active constituents of ergot were alkaloidal in nature. Very recently Barger (35) published the History of Ergot, an excellent review of the status at present. Since the isolation of these alkaloids in Europe by Tanret, Barger and Stoll, a great deal of information regarding the chemical and physiological nature of these alkaloids has been made available by these as well as other investigators. The different names which have been applied to these alkaloids, and also the conflicting reports concerning the pharmaco-dynamic activity of these various constituents and ergot galenicals have rendered the literature very confusing owing perhaps to results obtained from impure mixtures of the constituents and the great variations exhibited in the response of the various animals and methods used in pharmacological studies.

THE PHYSIOLOGICALLY ACTIVE CONSTITUENTS OF ERGOT.

The constituents of ergot which are responsible for the pharmaco-dynamic activity of its preparations may be conveniently divided into two groups, 1st, a series of alkaloids, and 2nd, a series of amines or amino bases. The crude drug contains appreciable quantities of a fixed oil, varying from 10 to 30 per cent, in addition to a coloring principle commonly known as sclererythrin.

The identity of these active constituents, with the chemical and pharmacological natures thereof, have been the subjects of intensive research for over a hundred years, and much remains to be done before even the more recent evidence set forth will be universally accepted as knowledge among the scientific investigators interested in ergot. However—although many phases remain disputed or unknown—all of the work has not been in vain.

At the present time it seems generally agreed that the alkaloids are the specific constituents responsible for the clinical activity of the drug (1). At least four different alkaloids have been identified with the drug, but the activity is believed to be due to but two, ergotoxine (3) and ergotamine (5). The other two, ergotinine (2) and ergotaminine (5) have been found to be relatively inactive by Kobert (7) and Spiro (5), respectively. The hydro-ergotinine of Kraft (4) has been shown to be identical with ergotoxine (6). Ergotoxine and ergotamine are active both orally and hypodermically.

It is further agreed that the proteinogenous amines identified with ergot are not responsible for the desirable clinical effects of ergot preparations. Indeed, they are regarded as being non-specific for the drug since they do not always occur in freshly collected material. Spiro and Stoll (5) and Stoll and Rothlin (9) have found their presence inconstant. The most important of these non-specfic amines are histamine, tyramine, acetyl-, and possibly other cholines, histamine being of the greater importance because of its greater activity and occurrence (1). Their activity by oral administration is doubtful (14).

The literature concerning the pharmaco-dynamic activity and chemical natures of all of these constituents has been reviewed by several investigators. A repetition is not necessary here. Reference is made to the reports of Barger and Dale (6), Broom and Clark (13), Clark and Broom (19), Edmunds and Hale (30), Forst and Weese (16), and the more recent work of Nelson and Pattee (1), Mendez (33), Rothlin (31) and Barger (35).

From all of this available experimental evidence it becomes readily apparent that any ergot preparation, in order to be of value for the purpose intended, *must contain significant amounts of the specific ergot alkaloids*, in a reasonably stable condition, and further, that these alkaloids must be present in defined amounts to make intelligent dosage possible. These requirements can be fulfilled only by the proper selection of methods of extraction and preparation and by employing a method for either chemical or biological standardization which will accurately estimate the alkaloidal content or physiological activity of the galenical.

THE PREPARATIONS OF ERGOT AVAILABLE TO THE PHYSICIAN.

At the present time, aside from innumerable proprietary preparations of ergot on the market, there exist two distinct types of ergot galenicals which have been accorded official recognition. The National Formulary, 5th Revision, hereafter called the N. F., recognizes a semi-solid extract prepared by extracting the drug with a chloroform water menstruum, with subsequent removal of supposedly inert material by precipitation with alcohol and evaporation of the excess menstruum at a moderate temperature. It has been designated as **Extractum Ergotae Aquosum** and is not required to be standardized in any way. In the trade it is commonly known as "ergotin" and is intended for oral administration. The U. S. Pharmacopœia, 10th Revision, hereafter called the U. S. P., recognizes "Fluidextract of Ergot," prepared by extraction of the drug with an acid-hydro-alcoholic menstruum. It is required by the U. S. P. to be physiologically standardized by the Cock's Comb Method therein described. It is suitable for oral administration only because of its acid and alcohol content and the presence of active and inert ingredients other than the specific ergot alkaloids.

THE METHODS EMPLOYED IN THE ASSAY AND STANDARDIZATION OF ERGOT PREPARATIONS.

Because of the complex chemical nature and the enormous differences in the physiological activity and potency of the active constituents of ergot and its preparations, it has been found impossible to accurately assay or standardize these preparations by chemical methods. Biological methods only can be depended upon to measure and standardize their activity. Of the numerous biological

methods which have been proposed only a few are conceded to be of value as assay methods and none of these are universally accepted. These methods, in the order of their usage or currently conceded value, are as follows:

1. The Cock's Comb Method, outlined and at present official in the U. S. P. Details of the method were published in 1927 by Gittinger and Munch (15) and in 1928 by Pattee and Nelson (28). It is reputed to measure the specific alkaloidal activity of the ergot preparation.

2. The Isolated Rabbit Uterus Method of Broom and Clark (13), and modifications (28), in which the strength of an ergot preparation is determined by the ability of the alkaloids to decrease the response of the isolated uterus of the rabbit to constant doses of epinephrine. It measures the specific alkaloidal activity of the preparations. The same principle has been applied to other tissues by Planelles (32), Rothlin (31) and others, with no particular advantages as applied to bio-assay adaptability, but of very great value in studies of the nature of ergot activity.

3. The Guinea-Pig or Cat Uterus (excised and *in situ*) Methods. These methods have been found to be of little value for ergot bio-assay purposes, because they measure the resultant effect of both the non-specific amines and the specific alkaloids (13).

4. The Pressor Methods to Cats or Dogs (13, 14, 26). These methods, though exceedingly meritorious in qualitative studies, are open to serious objections as bio-assay methods for ergot preparations because they, too, measure the resultant effect of both the amines and the alkaloids of ergot. Animal variation is also too great to permit of accurate quantitative information.

Thus, methods Nos. 1 and 2 are universally regarded as being the most accurate in estimating the clinical or specific alkaloidal activity of ergot preparations. Both have disadvantages but, in comparative studies using both methods, they have been found to yield practically identical results by Pattee and Nelson (28). Swanson (34), still more recently, reported similar results. Broom and Clark (13), Pattee and Nelson (28) and Swanson (34) agree that the Rabbit Uterus Method affords somewhat greater precision than the Cock's Comb Method but that the former is more difficult of technique and therefore requires more experience in the use of the method.

In spite of the fact that these investigators have found these two methods to be reasonably accurate in estimating the specific alkaloidal activity of Ergot preparations, the situation regarding available ergot preparations is far from being acceptable. In connection with his work in this laboratory, the author has examined by these two and other methods some hundreds of these preparations, prepared and distributed by the pharmaceutical manufacturers in both this country and abroad. This survey has revealed that statements of physiological potency and standardization upon their labels are, in fact, practically without meaning. Only a comparative few of the ampul preparations have been found to contain any alkaloidal activity whatever. The potency of the most important preparation of all, Fluidextract of Ergot, was found to vary from total inactivity up to as high as five times that specified by the U. S. P. and, to increase the gravity of the situation, the number not complying with the U. S. P. requirements was found to be vastly greater than the number of those which did. Strange to say, more were found to exceed the U. S. P. potency requirement than otherwise. A tabulation of the results of this survey will be published at a later date. The deplorable condition surrounding ergot cannot be attributed to the crude drug since none is admitted entry into this country unless it meets the U. S. P. requirements regarding potency, in addition to complying with the pharmacognostic

specifications. Obviously then, one or more of but three factors could be held responsible for the non-uniformity now existing; the unstable nature of the specific ergot alkaloids in the preparation (9), faulty extraction technique in manufacture, or, the possibility that the methods used in the biological assay and standardization of these galenicals are not accurate in all instances.

An attempt to clarify the present turbid situation has been the purpose of this investigation. Intensive studies involving the stability, nature and standardization of the various types of ergot preparations by the methods enumerated have been carried out, the results of which will be found in subsequent reports.

SUMMARY OF INVESTIGATIONS.

The following summarizes the results of the investigations:

1. The alkaloids of ergot produce bluing of the comb and wattles of cockerels, the intensity being proportional to dosage.

2. Histamine, and mixtures of the non-specific amines of ergot, produce in the absence of ergot alkaloids, a similar bluing, which is also proportional to dosage.

3. The addition of certain proportions of the amines of ergot to the alkaloids of ergot decreases the apparent potency as shown by the Cock's Comb Method. Assays by the Cock's Comb Method yield results which are too low in the case of freshly prepared fluidextracts high in amine content. After the amines are removed, the Cock's Comb test is thoroughly reliable and reasonably accurate in measuring the alkaloidal activity.

4. It is believed that histamine causes the greatest amount of interference. This amine, together with other amines and water-soluble principals, if present in sufficient quantities, as was found possible, will interfere with the assay of the ergot alkaloids by the Cock's Comb, Pressor, Guinea-Pig or Cat Uterus, or the original Broom and Clark Isolated Rabbit Uterus Methods.

5. A modification of the original Broom and Clark Method was found to yield results which were least influenced by these water-soluble amines.

6. A method has been devised which will detect and accurately measure the amine content of ergot preparations.

7. A method has been devised for the chemical removal of these interfering amines, thereby making possible an accurate estimation of the alkaloidal potency by any of the accepted bio-assay methods.

8. Extraction of powdered ergot with an aqueous menstruum removes principally amines and does not extract significant amounts of the active alkaloids. **Extractum Ergotae Aquosum** N. F. V and similar aqueous preparations, owe their activity to amines and are practically devoid of ergot alkaloids.

9. Extraction of powdered ergot with an acid hydro-alcoholid menstruum as in the U. S. P. fluidextract removes all of the amines and the alkaloids.

10. The amines were found to be less stable than the alkaloids in the menstruum of the U. S. P. fluidextract. The rapid amine deterioration results in an apparent increase in alkaloidal activity when tested by the Cock's Comb Method. The activity as shown by this method becomes stabilized after the amines have deteriorated (usually eight months or less). A slow decrease in strength then begins, depending upon storage conditions, due to deterioration of the alkaloids.

11. Regardless of variety or origin, almost every sample of ergot on the American market was found to contain an appreciable although varying amount of amine activity. A fairly large percentage contained enough amines to interfere seriously with the estimation of alkaloidal content by the Cock's Comb and other methods unless removed before assay.

The series of succeeding reports will embrace the following subjects in the order mentioned:

Part II. "A Biological Method for the Estimation of the Non-specific Amine Activity of Ergot and Its Preparations."

Part III. "A New Method for the Estimation of the Specific Alkaloidal Activity of Ergot and Its Preparations, Using the Isolated Guinea-Pig Uterus."

Part IV. "A Method for the Preparation of a Purified Fluidextract of Ergot."

Part V. "A Comparative Pharmacological Study of Crude Ergot and Fluidextract of Ergot, U. S. P. X," by:

A. The Cock's Comb Method.
B. The Isolated Rabbit Uterus Method of Broom and Clark, and Modifications.
C. The Pressor Method to Anesthetized Dogs.
D. The Isolated Guinea-Pig Uterus Method of Thompson.

Part VI. "A Pharmacological Study of Extractum Ergotae Aquosum, N. F., Fifth Edition," by:

A. The Cock's Comb Method.
B. The Isolated Rabbit Uterus Method of Broom and Clark, and Modifications.
C. The Pressor Method to Anesthetized Dogs.
D. The Isolated Guinea-Pig Uterus Methods of Thompson.

Part VII. "A Pharmacological Study of Purified Fluidextract of Ergot," by:

A. The Cock's Comb Method.
B. The Isolated Rabbit Uterus Method of Broom and Clark, and Modifications.
C. The Pressor Method to Anesthetized Dogs.
D. The Isolated Guinea-Pig Uterus Methods of Thompson.

Part VIII. "A Consideration of Standards for the Biological Assay and Standardization of Fluidextract of Ergot, U. S. P."

Part IX. "A Study of the Stability of Crude Ergot and Its Preparations: Changes Brought About by Aging."

Part X. "Discussion of Results; with Conclusions and Recommendations."

PART II. A BIOLOGICAL METHOD FOR THE ESTIMATION OF THE NON-SPECIFIC AMINE ACTIVITY OF ERGOT AND ITS PREPARATIONS.

The nature and identity of the physiologically active constituents of ergot were discussed in the first paper of this series (37). Experimental evidence shows that the ergot alkaloids are the constituents which are specific for the drug and responsible for the desired clinical effects of its preparations. The amines or amino-bases identified with the drug are regarded as being non-specific for ergot, as their presence has been found to be inconstant by Spiro and Stoll (5) and others (16). The author has found also that the non-specific amine activity of crude ergot varies enormously. Though a few samples were found to be practically free from active amines, most of those examined contained far too much amine activity to be disregarded.

A comparatively large number of amines or amino-bases, including histamine (ergamine), tyramine, acetyl and other cholines, agmatine, guanido-butylamine, and iso-amylamine, have been identified in Ergot. The literature(1, 16, 13), however, leads to a belief that only histamine, tyramine and possibly the cholines are sufficiently active, or have been shown to be present in adequate amounts to have any important share in the pharmaco-dynamic action of ergot preparations. Broom and Clark (13) have shown that the activity of B. P. Liquid Extract of Ergot is due almost entirely to the amines present, and that the U. S. P. Fluidextract of Ergot owes its activity to both groups of active principles—the alkaloids *and* the amines. Nelson and Pattee (28) state that "though no statement of the relative amounts of the two substances (histamine and tyramine) present in ergot preparations is available, it is usually accepted that more activity may be ascribed to the

histamine present than to the tyramine," because of the greater activity and occurrence of histamine.

In determining the amine activity of ergot or its preparations, it has not been found practical to use chemical methods because of the complexities encountered. Biological methods show more promise of adaptability, but results by these methods are often greatly influenced by the possible presence of the other group of active constituents—the specific alkaloids.

It is very difficult to separate quantitatively the two groups of pharmacodynamically active principles of ergot. Therefore accurate information on amine and alkaloidal activity may be obtained only by using methods that will measure the individual activity of the amine group, irrespective of the presence of the alkaloidal group, and vice versa. Up to the present time no suitable methods have been developed for the quantitative estimation of the amine activity of ergot preparations in the presence of the alkaloids, nor has a practical method been reported for the separation of the two groups of constituents, to make individual assay possible. The methods which follow were developed to supply this lack.

The biological assay methods generally used in the estimation of physiological activity of ergot and its preparations are of three general types, as follows:

(1) The Cock's Comb Method, and modifications.
(2) The Uterine Methods, with modifications:
    (*a*) Excised or Isolated, of Cats, Rabbits or Guinea-Pigs.
    (*b*) *In situ*, of Cats, Rabbits, Guinea-Pigs, etc.
(3) The Pressor Methods:
    (*a*) Systemic Circulation of Cats, Dogs, etc.
    (*b*) Pulmonary Circulation of Cats, Dogs, etc.

The Cock's Comb Method is reputed to measure the alkaloidal activity of the preparations (28, 29, 30), and is therefore of no value in estimating the amine activity in the presence of the alkaloids.

The uterus *in situ* methods measure the resultant effects of both the amines and the alkaloids (26, 13). The excised or isolated tissue methods are of two types: (1) Those in which the activity of the ergot preparation is observed directly; and (2) those in which the well-known antagonizing or vasomotor reversal effect of ergot alkaloids upon the response of the tissue to epinephrine is utilized (13, 19, 14, 28, 33, 31). In those of the first type, in which the activity of the preparation is observed directly upon the isolated uterine strips of cats or guinea-pigs, a resultant effect of the amines and alkaloids is observed, the individual constituents of both groups stimulating the tissue to contraction (1, 5, 8, 13). In those of the second type, involving the vasomotor reversal principle, the specific alkaloidal activity only is measured, as the amines do not have the the property of paralyzing the motor and inhibitory endings of the sympathetic to the action of epinephrine, the alkaloids alone being responsible for this effect (13, 31).

The Pressor Methods measure the resultant effect of all of the active principles of ergot. The alkaloids and tyramine produce a rise in blood pressure, while histamine produces a fall. The pressor effects of the amines are more transitory than those of the alkaloids, but, even so, an accurate quantitative estimation of either amine or alkaloidal activity of ergot preparations is practically impossible in many instances by this method.

Regardless of which of the pharmaco-dynamically active constituents of Ergot are responsible for the clinical activity of its preparations, all of them must be considered from the standpoint of biological assay, because it is possible for both the amines and the alkaloids to occur in sufficient amounts to manifest great activity by the currently used bio-assay methods. The author has developed a method which will measure the amine activity of crude ergot and its galenicals, irrespective of the specific alkaloidal content.

EXPERIMENTAL.

After many experiments involving the use of the various Pressor and Uterine Methods, a certain type of guinea-pig uterus was found adaptable for estimating the amine activity. Many of these biological methods have been described in the literature, but all were found to be inaccurate in the presence of the alkaloids. Using the isolated uterus of virgin guinea-pigs weighing from 200 to 300 grams, it was observed that the amines (histamine and tyramine) and the alkaloids (ergotamine and ergotoxine) all stimulated the tissue to marked contractions. In testing mixtures of these amines and alkaloids, the resultant effect of both groups was observed. In marked contrast to this observation, isolated uterine strips of large mature guinea-pigs, taken several weeks post-partem, were stimulated to contraction by the amines of ergot under certain

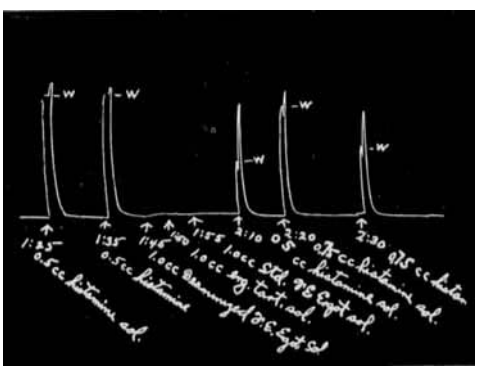


Fig. 1.—The response of the isolated uterus of the mature, non-pregnant guinea-pig to histamine and ergot alkaloids. Note the constancy of the response to histamine before the addition of the alkaloidal solutions to the baths; the ineffectiveness of the alkaloids in inducing tonic contractions; and the inhibiting influence of the alkaloids upon the histamine response.

conditions, but the alkaloids seemed to have no perceptible effect except to increase the tonus in spontaneous rhythmic contractions.

The first two contractions shown in Fig. 1 were produced by identical concentrations of histamine. Following these two contractions of equal magnitude, at 1:45 an amine-free Fluidextract of Ergot produced no contraction, although it was known to be rich in ergot alkaloids. At 1:50 a dose of ergotamine tartrate, a salt of one of the most active of the ergot alkaloids, produced no contraction. At 1:55 1.0 cc. of U. S. P. X Standard Fluidextract, dilution 1 to 5, produced no perceptible effect. Thus, the alkaloids of ergot, or preparations free from active amines but known to contain significant quantities of ergot alkaloids, failed to produce contractions of this type of isolated uterus. Figure 1 also shows the following interesting phenomenon. At 2:10, following the addition of the three alkaloidal preparations to the bath, a repetition of the original dose of histamine produced a contraction only half as great as that produced before the alkaloidal preparations were added. At 2:20 a 50 per cent increase of the histamine dose produced a contraction greater than that produced by the dose immediately preceding it, but less than that produced by the smaller dose before the strip was subjected to the action of the ergot alkaloids. Repeating the higher dose at 2:30 produced less effect than that produced at 2:20. Thus it is shown that *the alkaloids decrease the response of the isolated guinea-pig uterus of this type to histamine* in a manner analogous to the vasomotor reversal effect of ergot alkaloids upon the epinephrine response of various tissues. The indications are, therefore, that histamine acts upon essentially the same mechanism as epinephrine, although epinephrine causes the guinea-pig uterus

to relax instead of to contract. This hitherto unreported "ergot-alkaloid-histamine" reversal phenomenon has been utilized in the development of a method for the estimation of the alkaloidal activity of ergot preparations, which will be described in the third paper of this series. Pressor studies of this reversal will also be published in a later report.

As in the case of the ergot alkaloid-epinephrine reversal, the ergot alkaloids must act for some time before the histamine response is diminished. Hence the method may be utilized in the estimation of the amine activity of ergot preparations, even though the alkaloids also are present. An accurate estimation of the amine activity of crude ergot or its preparations may be obtained by observing in detail the method and procedure which follows:

METHOD.

MUSCLE.

The uteri of guinea-pigs weighing 500 to 800 grams, taken several weeks post-partem, are the most satisfactory. Uteri of smaller guinea-pigs, either nulliparous or multiparous, usually prove unsuitable, as they are often stimulated to contraction by both the amines and alkaloids of ergot. Pregnant uteri and those taken shortly after parturition are not suitable. Small, thin-walled uteri, as well as those showing diseased conditions, also must be discarded. As it is often very difficult to detect pregnancy in the animals, especially in the earlier stages, it has been found advantageous to segregate the female pigs shortly after parturition. Several weeks thereafter their uteri are usually suitable. Lactating animals are unsatisfactory. In general, the type of guinea-pig uterus best suited to this method is comparable to the type of rabbit uterus recommended for use in the Broom and Clark Rabbit Uterus Method for the estimation of ergot alkaloids (13, 28). From the larger guinea-pig uteri, eight to twelve suitable strips may be obtained, each horn being divided into two or three cross sections, which, in turn, are halved longitudinally by two parallel cuts, one directly along and one directly opposite the mesenteric attachment. The uteri may be kept on ice for three or four days in physiological salt solution, but it is best to use them before such a time has elapsed.

APPARATUS.

The apparatus is of the type usually employed for isolated tissue work, with two or more glass chambers of 50 cc. or 100 cc. capacity, mounted in a constant temperature bath, arranged to fill and empty from below. The smaller suspension chamber would save on the use of Locke's solution and would undoubtedly be fully as suitable as those of 100 cc. capacity. Those of 100 cc. capacity were used in this work. Dale and Laidlaw (20), Smith and McCloskey (21), and Pattee and Nelson (28), describe this apparatus in greater detail.

METHOD FOR ESTIMATING NON-SPECIFIC AMINE ACTIVITY.

The similar uterine strips are suspended, one pair at a time, in separate chambers in oxygenated Locke's solution at 38° C. and arranged to record on a slow-moving kymograph in the usual manner. The tension on the strip is important, and can be determined by experience only. A dose of histamine which will produce a pronounced, but submaximal contraction for each strip is then determined. When the strips, after several trials, are found to produce contractions of constant submaximal magnitude from a given concentration, at a constant time interval between doses, the amine activity of the ergot preparation is ascertained by comparison. After each dose the strips are washed and the chambers refilled with Locke's solution. The use of two strips provides a check as well as a time-saving factor. If appreciable quantities of ergot alkaloids are present in the unknown, as is invariably the case in Fluidextract of Ergot, the first trial of the unknown on each strip is all that may be considered significant, because any alkaloids present diminish the amine response. If a certain sub-maximal dose continues to give constant contractions, as is often the case with aqueous extracts, it is indicated that active ergot alkaloids are not present. The amine activity is expressed in terms of histamine.

It is absolutely essential that all doses be added to the baths at definite time intervals. Exactly 10 minutes between each dose has been found satisfactory in most instances, although some uteri will contract and relax sufficiently in a shorter time. Whatever time interval is used, it must remain constant between each dose throughout the assay. Figure 2 shows the constant response to graded dosages obtained when a definite time interval is carefully observed.

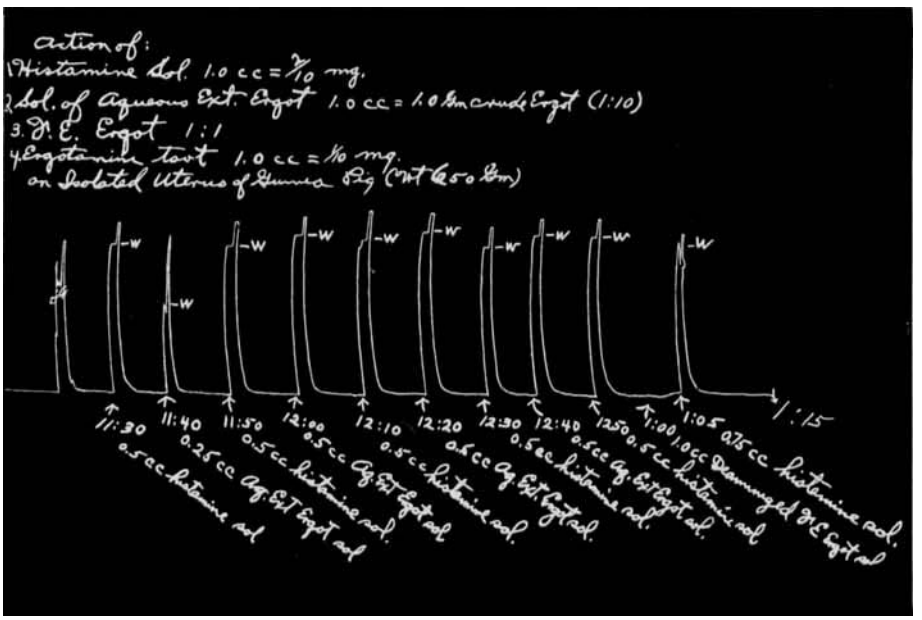


Fig. 2.—A determination of the non-specific amine activity of an ergot preparation by the isolated guinea-pig uterus method described. Note the constancy of the response to histamine and the ergot preparation until 12:50; showing the absence of ergot alkaloids but a pronounced amine activity. Note also the absence of response to the amine-free alkaloidal ergot preparation at 1:00, and the inhibiting influence of this alkaloidal preparation upon the response of the uterus to the increased dose of histamine at 1:05. The "W" indicates wash.

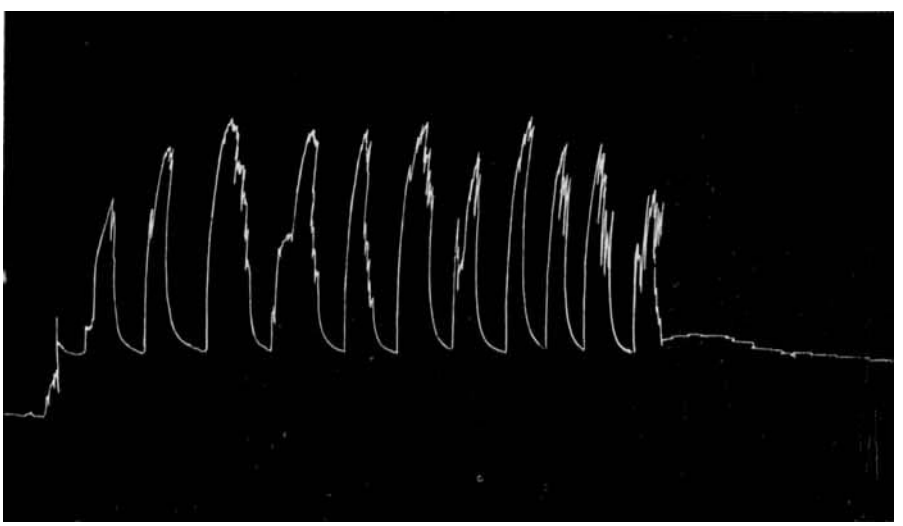


Fig. 3.—The variable response of the guinea-pig uterus to constant doses of histamine observed when an improper tension on the strip is used. The tension employed is of paramount importance.

The weighting of the levers is of paramount importance. It is impossible to state the amount of tension to be employed, as each strip will differ in this respect. In general, the levers must be weighted sufficiently to obviate appreciable spontaneous rhythmic contractions, but not so heavily as to cause symptoms of fatigue in the muscle. When the strips are found to react according to the dose added to the bath, and *constantly* to a given dose, they are ready for assay purposes.

If insufficiently weighted, the magnitude of contraction will not distinguish between varying doses. If weighted too much, the magnitude of contraction diminishes with each succeeding dose, when the doses are constant, or even when the dose is increased. Figure 3 shows the irregular response of a strip to constant dosage of histamine when improperly weighted.

The following is the composition of the Locke's solution that has been used in this work:

	Grams.
Sodium chloride	9.0
Potassium chloride	0.42
Magnesium chloride	0.005
Calcium chloride	0.24
Sodium bicarbonate	0.50
Dextrose	0.50
Glass-redistilled water to make	1000 cc.

This Locke's solution is freshly prepared each day from stock solutions of 20-fold concentration, exclusive of the sodium bicarbonate and dextrose. The sodium bicarbonate and dextrose are weighed out each time as required.

The oxygen used is passed through an aqueous 4 per cent sodium bicarbonate solution, and then conducted to the tissue chambers, where it is evolved through the Locke's solution surrounding the tissue. The rate is kept at approximately 20 bubbles per minute.

DETERMINATION OF NON-SPECIFIC AMINE ACTIVITY OF CRUDE ERGOT: PREPARATION OF TEST EXTRACT.

Grind the crude ergot and de-fat or de-grease as directed in the U. S. P. for Fluidextract of Ergot. A 50-gram portion is convenient. After the benzine odor has left the drug, add enough 10 per cent sodium bicarbonate solution to render the powder evenly and distinctly moist and to keep it so during 2 to 4 hours' maceration in a refrigerator. Then pack the moist drug loosely in a small glass percolator provided with a glass stop-cock. Add cold distilled water to the percolator, and, when the liquid begins to drop from the lower orifice, interrupt percolation and let maceration proceed for an additional 6 hours in a refrigerator. Allow the percolation to proceed at a rate of 15 drops per minute until a small portion directly from the percolator is found to be inactive on the guinea-pig uterus by the method previously described.

The amine activity of crude ergot, as well as the rate of extraction of the drug, varies enormously. It is therefore practically impossible to specify the amount of percolate to be obtained. All operations must be carried out at refrigeration temperature and the percolate must be stored in a refrigerator until the assay has been completed. At higher temperatures fermentation and putrefaction develop, resulting in increased amine activity. The percolate or aqueous extract thus prepared will be found to be practically devoid of active ergot alkaloids.

Knowing the volume of percolate collected from the specified amount of drug used in the assay, determine the amine activity by the guinea-pig uterus method described, expressing the result in terms of milligrams of histamine per gram of ergot or as the percentage of amine activity in terms of histamine.

Figure 2 shows an example of the estimation of the amine activity of a crude ergot by the methods described. Note that the uterine strip is shown to be unresponsive to the alkaloidal preparation at 1:00. After the alkaloidal dose, however, a 0.75-cc. dose of histamine solution at 1:05 produced a contraction no greater than the 0.5-cc. dose originally produced, showing partial paralysis of the uterine strip by the alkaloids to the effect of histamine.

DETERMINATION OF NON-SPECIFIC AMINE ACTIVITY OF SOLID, SEMI-SEMI OR LIQUID AQUEOUS EXTRACTS OF ERGOT.

Triturate an exact weight of the solid or semi-solid extract in a glass or agate mortar with a definite amount of Locke's solution (1:25 is a convenient concentration) until the extract is dispersed. (This applies only to the solid or semi-solid extracts, the others already being in a state of solution.) Carefully but completely neutralize any acidity by the cautious addition of

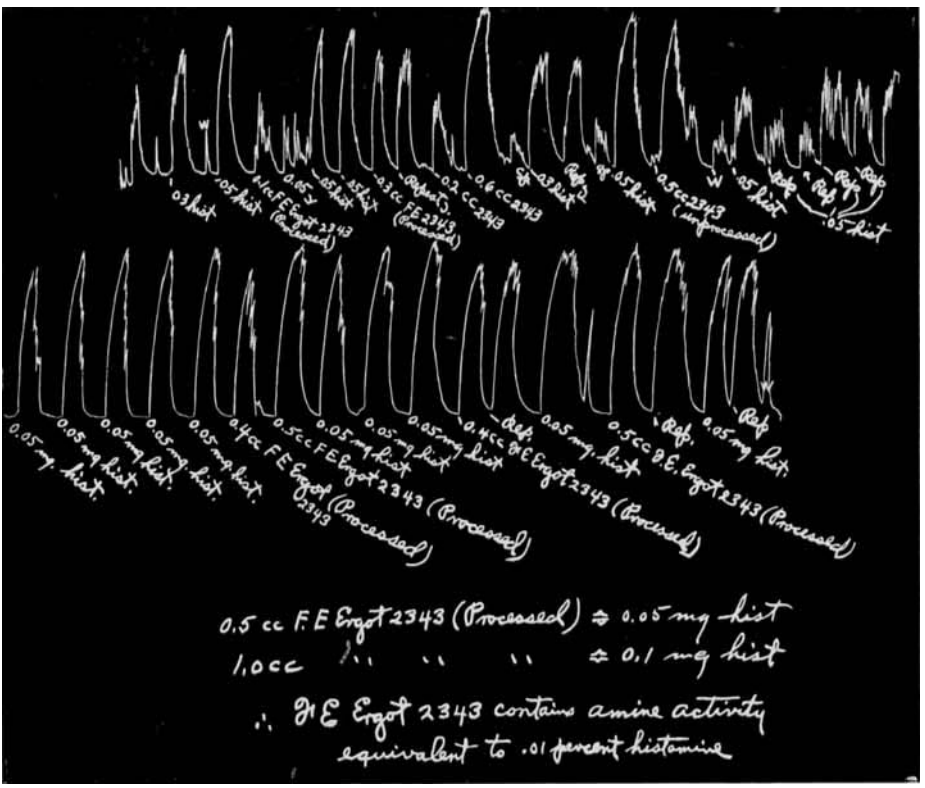


Fig. 4.—The response of two similar strips of isolated guinea-pig uterus. The upper tracing shows the effect of histamine and a Fluidextract of Ergot before and after being processed according to the method described. Note the constancy of response to the processed Fluidextract in varied doses as compared to the inhibited response to histamine after the unprocessed Fluidextract was added. The inhibition was produced by the interfering alkaloids present in the unprocessed preparation. The lower tracing shows a typical estimation of the non-specific amine activity of the same U. S. P. Fluidextract by the method recommended. Note the absence of alkaloidal interference as shown by the constant response to both the histamine standard and the processed Fluidextract.

powdered sodium bicarbonate, and filter. Do not bring up to volume by adding more Locke's solution after filtration.

Determine the amine activity by the guinea-pig uterus method as described for crude ergot. Results are conveniently expressed as percentage of amines calculated as histamine. The amine activity of all ergot preparations varies greatly. In determining this activity, the doses and concentrations must be varied according to the activity found. No alkaloidal interference should be encountered if this method is carefully followed.

DETERMINATION OF NON-SPECIFIC AMINE ACTIVITY OF FLUIDEXTRACT OF ERGOT AND OTHER ALCOHOLIC PREPARATIONS OF ERGOT.

Pippette 10 cc. of the preparation into a small beaker and dealcoholize by using a warm-air blast (a barber's hair dryer serves admirably). Carefully neutralize any acidity by the cautious addition of powdered sodium bicarbonate. Bring up to the original volume with Locke's solution, mix thoroughly and filter.

Determine the amine activity by the guinea-pig uterus method as directed for crude Ergot. The amine activity is conveniently expressed in percentage, calculated as histamine. These preparations will also be found to vary enormously in active amine content, some being devoid of such activity while others contain more than the equivalent of one milligram of histamine per cubic centimeter of preparation. Figure 4 shows an assay of the amine activity of a sample of U. S. P. Fluidextract of Ergot. Note the absence of alkaloidal interference, shown by the constancy of the magnitude of contraction to graded dosage of the processed preparation.

SUMMARY.

A type of guinea-pig uterus is described which, under specified conditions, is stimulated to contraction directly by the non-specific amines of ergot, but is not so affected by the specific ergot alkaloids. The alkaloids increase the tonus in rhythmic contraction of the muscle, but do not stimulate prompt tonic contractions directly.

The alkaloids of ergot were found to inhibit the response of the guinea-pig uterus to histamine in a manner analogous to the well-known vasomotor reversal relation between ergot alkaloids and epinephrine upon various tissues.

A method is described for the estimation of non-specific amine activity of ergot and its preparations, including methods for the preparation of the test solutions which largely obviate alkaloidal interference. Histamine was used as the standard for comparison because it is responsible for the greater amount of the amine activity of ergot, and, further, because it is readily available.

PART III. A METHOD FOR THE ESTIMATION OF SPECIFIC ALKALOIDAL ACTIVITY OF ERGOT PREPARATIONS UPON THE ISOLATED UTERUS OF THE GUINEA-PIG.

The recent literature on ergot leads to a belief that the specific alkaloidal activity of ergot is due to ergotamine and ergotoxine and that these two alkaloids are identical as to pharmacological and clinical action, although they differ somewhat in chemical structure and physiological potency (13, 1, 28).

The chemical studies of Kraft, Tanret, Barger and Stoll have made these alkaloids available in the pure state. By the successful isolation of these alkaloids of ergot, pharmacological and clinical studies on them were made possible. The reports of these studies tend to establish that the ergot alkaloids are specific for the drug and responsible for the desirable clinical effects of ergot preparations, while the amines (histamine, tyramine, etc.) are non-specific and therefore relatively unimportant from the clinical standpoint.

Because of the great amount of experimental evidence thus set forth, the present tendency in the biological assay and standardization of ergot preparations is to employ methods that measure the specific alkaloidal activity, disregarding

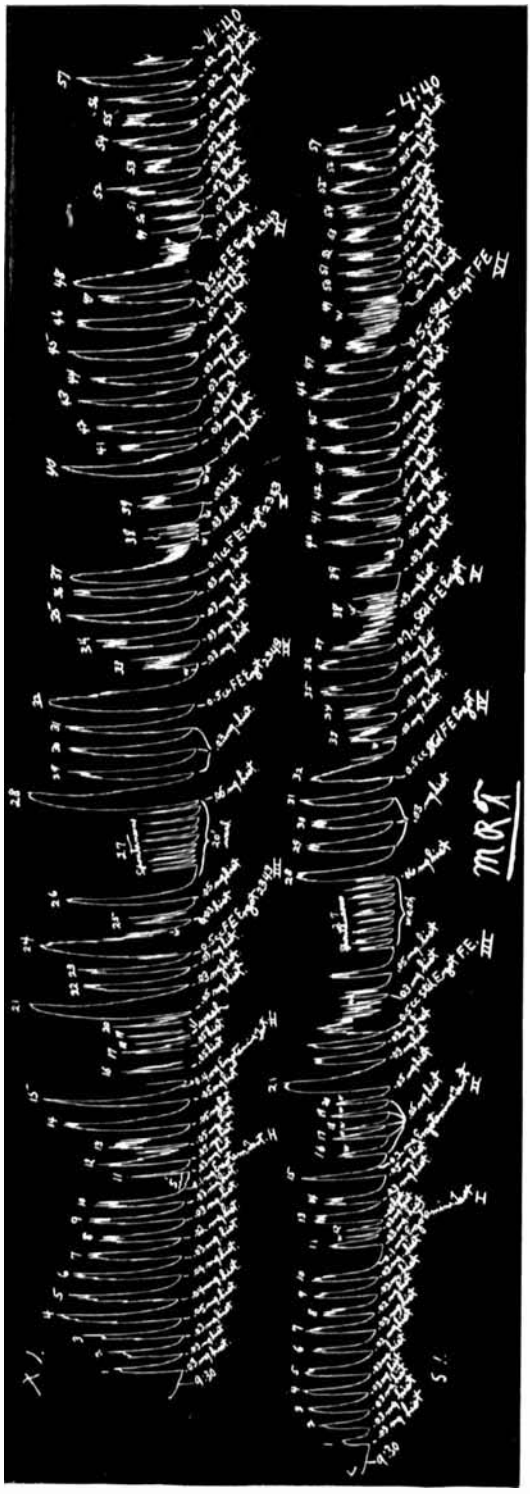
completely the presence of the non-specific amines. Just what part the amines, such as histamine and tyramine, play in the clinical activity of ergot preparations is not definitely known, although histamine, the most important of them, is reported to be practically without effect when administered orally (14) at least in the amounts ordinarily found in these preparations. Both histamine and tyramine manifest great activity when administered hypodermically, however, and it is possible that they play a part in the action of preparations intended for hypodermic use. It is generally known that the ergot alkaloids are effective *both orally and hypodermically* and that they are specific for the drug, thereby establishing their greater importance.

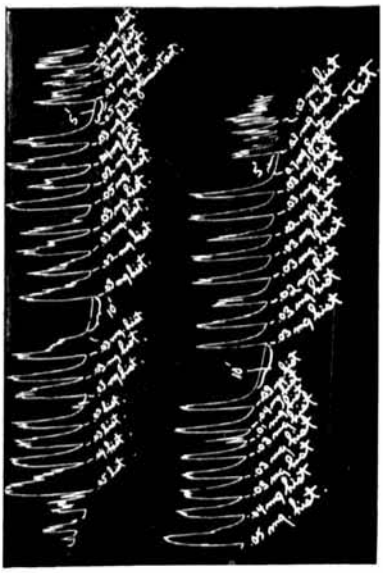
Of the many methods proposed for the biological standardization of the specific alkaloidal activity of ergot preparations, only two have shown sufficient reliability in application to warrant general recognition. These are the so-called Cock's Comb test, official in the U. S. P., and the Isolated Rabbit Uterus Method of Broom and Clark (13). Pattee and Nelson (28) found these two methods to yield practically identical results in estimating the alkaloidal activity of ergot preparations. Broom and Clark (13) were unable to get accurate results by the Cock's Comb Method because of variation in susceptibility of cocks. Edmunds and Hale (30) found the Cock's Comb Method to be reasonably accurate. In the writer's experience, the two methods yield comparable results in most instances, but great variations were observed in some cases. The results obtained in comparative studies of these two and other methods will follow in other papers of this series.

A method for the estimation of the specific alkaloidal activity of ergot preparations has been developed which, it is believed, is worthy of consideration, since it clearly shows the difference between the amine and alkaloidal activity of ergot upon the isolated uterus of the guinea-pig. This method is comparable to the Isolated Rabbit Uterus Method of Broom and Clark (13), with the exception of the smooth muscle employed and the substance used for stimulating the tissue to contraction. Bio-assayists experienced in the use of the Rabbit Uterus Method have found considerable difficulty in obtaining rabbits of the type necessary, as pointed out by Pattee and Nelson (28) and Swanson (34). Guinea-pigs are comparatively plentiful and are cheaper than other experimental animals suitable for the purpose. The uterus of the guinea-pig is utilized in the new method.

The well-known "ergot alkaloid-epinephrine reversal" cannot be applied to the guinea-pig uterus because epinephrine causes relaxation instead of contraction of this tissue. It has been shown that a similar relation exists between the ergot alkaloids and histamine (Part II). Histamine was shown to cause definite tonic contraction of strips of isolated guinea-pig uterus of a specified type. This contraction could be inhibited or abolished by allowing the ergot alkaloids to act upon the tissue for a short time. A certain definite concentration of ergot alkaloids is required to produce this inhibition or reversal and therefore this reaction can be utilized to estimate the quantity of alkaloidal activity in ergot preparations.

The method has two distinct advantages over the Isolated Rabbit Uterus Method. Suitable guinea-pigs are more readily available than suitable rabbits, and the uterine strips of the guinea-pig recover from the effects of the ergot alkaloids rather quickly, thereby permitting several trials upon a single strip (Fig. 7).





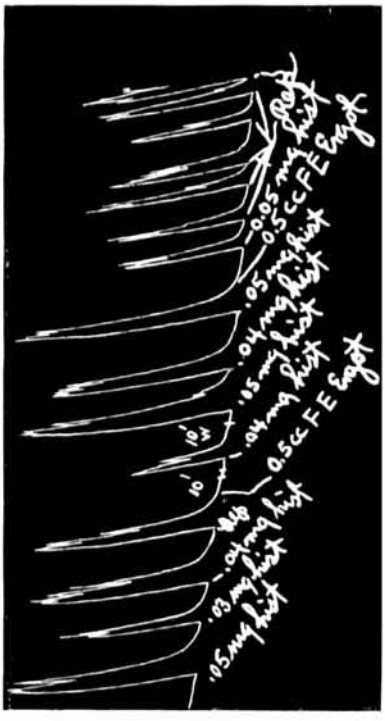


Fig. 5, below, on left. Fig. 6, below, on right. Fig. 7, top. For legends see next page.

Fig. 5.—The response of the isolated guinea-pig uterus to histamine, and a U. S. P. Fluidextract of Ergot which was practically devoid of amine activity. The Fluidextract is shown to contain sufficient alkaloidal activity to appreciably inhibit the normal response to histamine.

Fig. 6.—Showing the constant response of similar strips of isolated guinea-pig uterus to histamine up to the point of the administration of ergotamine tartrate. By permitting this alkaloid to act upon the strips for five minutes, the response to histamine was appreciably inhibited in each case.

Fig. 7.—The upper and lower tracings show the response of a single pair of similar strips of guinea-pig uterus. The first few contractions of each strip illustrate the necessity of repeating the doses of histamine until constant response to a given concentration is obtained. Following this, each strip was subjected to six different applications of alkaloidal ergot preparations during a period of over seven hours.

In each instance the alkaloids were permitted to act for a time sufficient to cause a definite inhibition of the histamine response. Recovery from the alkaloidal effect can be noted after each application. The strips were functioning satisfactorily when the experiment was discontinued at the end of seven hours.

THE ERGOT ALKALOID-HISTAMINE REVERSAL.

The first evidence of this reversal was observed in pressor studies of ergot preparations. This led to the supposition that it should be possible to produce further evidence of such a reversal upon uterine muscle. Experimental results (Part II) show the inhibitory action of ergot alkaloids upon the response of the isolated guinea-pig uterus to histamine or ergot amines. Broom and Clark (13) in their Fig. 4, actually show this reversal relation between ergot alkaloids and amines upon the isolated guinea-pig uterus, although they offer an explanation with which the writer does not entirely agree. They show the effect of a B. P. Liquid Extract upon one horn and of a U. S. P. Fluidextract of Ergot upon the other horn of the isolated uterus of the same guinea-pig. The response of the muscle to the B. P. extract remained constant "because it depends entirely on the histamine content." The writer agrees that this preparation contains practically no active alkaloids, but, because of experimental pressor studies, believes that the activity is due to a mixture of amines rather than to histamine alone, although histamine is the most active and predominating amine regarding activity. The response of the muscle to U. S. P. Fluidextract of Ergot, in the same illustration, "becomes less on repetition because it depends largely on the alkaloidal content of the ergot." This, in the writer's experience, is true only in part. Since, as Broom and Clark state, both of these preparations were made from the same batch of ergot, each should contain comparable quantities of active amines. The B. P. and U. S. P. processes of manufacture, if the same batch of drug is used in each, yield preparations which are similar with respect to amounts of non-specific amine activity, differing only in the specific alkaloidal content. The U. S. P. Fluidextract always contains more of the alkaloids than the B. P. preparation. This has been shown repeatedly by the writer. Broom and Clark (13) explain the diminishing response from the repeated administration of the U. S. P. Fluidextract to the isolated uterus of the guinea-pig as follows: "* * * the amines present in ergot produce an effect as great or greater than that produced by the ergot alkaloids. The ergot alkaloids, moreover, can only excite the uterus once and after the first application of ergotamine the uterus will not respond to a second administration although its excitability to histamine is unaltered. The ergot extracts were found to vary as regards the constancy of the reaction they produced; submaximal doses of the B. P. preparations produced constant response repeatedly, whereas with submaximal doses of the U. S. P. preparations the first application produced the greatest response. This effect is shown in Fig. 4. Apparently in the case of the U. S. P. preparations about half the action on the guinea-pig's uterus is due to its alkaloidal content and about half to its amine content. In the case of the B. P. preparation the whole action is due to its amine content."

These investigators do not state the type of guinea-pigs used in their work. Experiments involving different types of guinea-pig uteri definitely show that this is a vital factor. The isolated uteri of young, immature guinea-pigs usually are stimulated to contraction by both the alkaloids and the amines of ergot, histamine being decidedly most active in this respect. The

resultant effects of mixtures of alkaloids and amines were found to be similar to that described by Broom and Clark (13) for U. S. P. Fluidextract of Ergot. If the isolated uteri of larger, mature guinea-pigs are employed, however, the contractions produced are due almost entirely to the amines present. In the case of B. P. Liquid Extract or the N. F. V Aqueous Extract, constant response of the tissue is observed because the activity of these preparations depends almost entirely upon the amine content. Fluidextract of Ergot contains all of the amines and alkaloids that were present in the parent drug. The first application of this preparation to the uterine strip results in a contraction whose magnitude depends upon the amine content of the fluidextract. Upon repetition of the application the response diminishes, the lessening of the response depending upon the proportion of alkaloids and amines present and the time the preparation is permitted to act upon the tissue before replacing the poisoned Locke's solution with fresh Locke's solution. This diminishing effect is shown in Fig. 1, Part II of this manuscript, and is explained by the "ergot-alkaloid-histamine" reversal therein described. This consists essentially in the ability of ergot alkaloids to inhibit or abolish the response of the guinea-pig uterus to histamine or ergot amines in a manner analogous to the well-known "ergot-alkaloid-epinephrine" reversal upon the rabbit uterus.

The utilization of the "ergot-alkaloid-histamine" reversal upon the guinea-pig uterus in estimating the alkaloidal activity of ergot preparations would at first seem impossible, as all of these preparations usually contain variable quantities of histamine as well as other amines. Therefore, when the ergot preparation is applied, the presence of histamine or other amines of ergot would increase the ultimate amine concentration of the bath, causing an abnormally great contraction of the uterus when the histamine effect was observed after the alkaloids had exerted their definite inhibiting effect. Experimental studies have shown that the natural amine content of ergot preparations causes no greater interference in this method than in the Rabbit Uterus Method, in which the test contraction is induced by epinephrine. The amine interference is obviated in this Isolated Guinea-Pig Uterus Method in the same way that the same interference is obviated in the Isolated Rabbit Uterus Method as modified by Pattee and Nelson (28). These investigators state that by sufficient washing of the strip of rabbit uterus after the ergot preparation has acted upon it for a definite length of time, but before the final epinephrine response is observed, the interfering amines are removed, thereby permitting the epinephrine to produce its true alkaloid-inhibited response. They also show definitely that the amines may be present in amounts sufficient to increase the ultimate epinephrine response if the washing technique is not observed. By observing the same technique the amine interference is obviated in the Guinea-Pig Uterus Method also.

METHOD.

The method is based upon the ability of the active alkaloids of ergot to inhibit or abolish the response of the isolated guinea-pig uterus to histamine (Fig. 5) in a manner analogous to the "ergot alkaloid-epinephrine" reversal principle utilized in the Isolated Rabbit Uterus Method of Broom and Clark (13). The method is carried out in the same manner as the Broom and Clark Isolated Rabbit Uterus Method (13, 28) except that similar strips of guinea-pig uterus are used instead of similar strips of rabbit uterus, and histamine is used instead of epinephrine to induce the contractile response.

APPARATUS AND MUSCLE.

The apparatus, Locke's solution, and smooth muscle employed in this method are the same as those used in the method described for the estimation of the non-specific amine activity of ergot and its preparations (Part II).

TECHNIQUE.

The uteri are obtained from mature guinea-pigs weighing from 500 to 800 grams, taken several weeks post-partem. Segregation of proper animals immediately after parturition largely obviates obtaining unsuitable uteri. The animal is killed by a blow on the head and the uterus is carefully dissected out in the usual manner, removing fallopian tubes, ovaries, etc. From the larger uteri, eight to twelve suitable strips may be obtained, each horn being divided into two or three cross sections, which, in turn, are halved longitudinally by two parallel cuts, one directly along and one directly opposite the mesenteric attachment. These strips are immersed in physio-

logical saline solution and kept on ice. In this condition they may be kept for 3 or 4 days, but it is preferable to use them before so long a time has elapsed. It has been the practice of the writer to make but one of the longitudinal cuts along each cross section before the section is to be used. When the section is to be used the other cut is made, dividing the section into two similar strips. This insures the use of similar, instead of dissimilar, strips.

Two similar strips are then mounted in separate chambers of the apparatus and arranged to record in the usual manner. The tension used is of paramount importance. It must be sufficient to produce relaxation and prevent appreciable spontaneous rhythmic contractions but not so great as to prevent a good contraction or cause the strip to show symptoms of fatigue (as evidenced by a diminishing response to a constant dose of histamine) in repeated application of histamine. Each strip used is a problem unto itself in this respect. The tension to be used can be determined only by experience. When properly weighted the spontaneous contractions are not in evidence, but a prompt contraction is produced by the addition of a proper concentration of histamine. The poisoned Locke's solution is replaced with fresh solution after each dose as soon as the maximum amplitude of the contraction is attained.

As shown in Fig. 7, the response of the strips to constant doses of histamine usually increases during the first few trials. In the writer's experience it is of advantage to let the strips stand for half an hour before testing their response to histamine. The response will then be more constant than it would if histamine were introduced immediately after mounting. The dose of histamine which will produce a pronounced but submaximal contraction is then determined by trial. This dose is repeated until the response of the strip becomes constant. After waiting twenty minutes, the constancy is again observed. Having thus determined the dose necessary and the constancy of response to this dose, the strips are in readiness for testing the alkaloidal activity of the ergot preparation.

The standard, which may be either the U. S. P. Standard Fluidextract supplied by this laboratory or a solution of one of the specific alkaloids, is added to one bath and the preparation to be tested is added to the other. In each case, the time of the addition of each is accurately recorded. At this point an indication of the non-specific amine activity of the preparation may be obtained. If the amine content is low, a low contraction will result; if high, the contraction will be correspondingly great. Occasionally preparations will be found to contain enough amines to cause a maximal contraction when they are added to the bath (Fig. 7). In any case, however, the strip gradually relaxes. The ergot preparation is allowed to act on the strips exactly fifteen minutes in each case, after which the strips are washed. After another three minutes the previously determined dose of histamine is repeated. When its effect has been obtained it is washed off and in a few minutes repeated as a check, all factors being carefully kept identical for each strip. Similarly, at definite time intervals of a few minutes, the effect of further repetitions of the same histamine dose is observed. The potency of the preparation is determined by a comparison of the percentage inhibition produced by the unknown with the percentage inhibition produced by the standard in a manner identical with that recommended by Pattee and Nelson (28) in carrying out the Broom and Clark Method. The dose of ergot should be sufficient to reduce the histamine response 25 to 50 per cent. Further reduction is hardly significant, as spontaneous rhythmic contractions of low magnitude frequently develop, making the exact inhibition difficult to determine. After several washings the uterus soon recovers from the effect of the ergot alkaloids. When the histamine response again becomes constant, as it usually does within half an hour, another trial of the ergot preparation may be made. The effect of the repetition of application of ergot alkaloids and recovery of the similar strips of guinea-pig uterus is shown in Fig. 7. In this experiment the effects of six different trials with alkaloidal ergot preparations were observed over a period of more than seven hours, at the end of which time each strip was still functioning satisfactorily.

It will be noted in the illustrations that much higher concentrations of ergot alkaloids are necessary to appreciably inhibit the histamine response of the guinea-pig uterus than are required to diminish the epinephrine response of the rabbit uterus. The inhibition of the histamine response depends upon two factors, the concentration of ergot alkaloids employed and the length of time the alkaloids are permitted to act upon the strips. In general, the concentration of the ergot alkaloids employed bears an inverse relation to the time of action necessary to produce a definite inhibition. Therefore, increasing the time of alkaloidal action from 5 or 10 minutes

(as used in the Broom and Clark Method) to 15 or 20 minutes permits the effective concentration of ergot alkaloids to be lowered.

It is impossible to state the concentration of ergot alkaloids or of histamine to be employed because of the variations in uteri. The illustrations give approximate indications of concentrations when 100-cc. tissue baths are used, although in these experiments the time interval used was varied. Table I shows typical results obtained in an assay:

TABLE I.—RESULTS OBTAINED FROM SIMILAR STRIPS OF THE SAME GUINEA-PIG UTERUS.

	Commercial sample Fluidextract of Ergot No. 6.		Standard Fluidextract of Ergot.	
Trial no.	Dose (in 100-cc. baths). Cc.	Inhibition, per cent.	Dose (in 100-cc. baths). Cc.	Inhibition, per cent.
1	0.5	>60.0	0.5	40.6
2	0.25	42.1	0.5	44.2
3	0.25	44.7	0.5	46.6
4	0.2	35.8	0.5	44.5
5	0.15	<30.0	0.5	47.2

> More than. < Less than.

The results in Table I show that 0.25 cc. Fluidextract No. 6 is approximately equivalent to 0.5 cc. of Standard Fluidextract Ergot. In other words Fluidextract Ergot No. 6 is approximately twice the strength of the Standard.

The results obtained by this isolated Guinea-Pig Uterus Method agree in most instances with those obtained by the Cock's Comb and the Broom and Clark Rabbit Uterus Methods. Comparative studies of these three methods will be given in another paper of this series.

SUMMARY.

Studies involving the use of the isolated uterus of the mature, non-pregnant guinea-pig have shown that the specific alkaloids and the non-specific amines of ergot act in a decidedly dissimilar manner. The pure alkaloids increase the tonus in rhythmic contractions but are relatively ineffective in stimulating prompt tonic contractions. The non-specific amines (histamine and aqueous extracts of ergot containing essentially nothing but histamine, tyramine, etc.) are very active in inducing prompt tonic contractions. The tonic contractions produced by histamine or mixtures of the non-specific amines of ergot can be inhibited or abolished by permitting the specific ergot alkaloids to act upon the muscle for a time.

It was observed further that the inhibition of the amine response of the isolated guinea-pig uterus by the ergot alkaloids depended upon the concentration of ergot alkaloids used and the time the alkaloids were permitted to act.

The method for the estimation of alkaloidal activity of ergot preparations here described depends upon this "histamine-ergot alkaloid" relationship upon the isolated guinea-pig uterus.

(*To be continued*)

ABSTRACT OF PAPER, SCIENTIFIC SECTION.

"Studies of the Analysis and Chemistry of Neoarsphenamine. II. Studies on the Chemistry of Sodium Formaldehyde sulphoxylate and Their Relation to the Structure of Neoarsphenamine," by A. E. Jurist and W. G. Christiansen.

The stability and reactions of sodium formaldehyde sulphoxylate in acid and alkali have been studied and its structure derived from these results. Thus the structure of Neoarsphenamine has been developed and the existence of at least two, and perhaps three, tautomeric forms is indicated.