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RESEARCH DEPARTMENT OF THE CHEMICAL AND PHARMACEUTICAL LABORATORIES,
E. R. SQUIBB AND SONS, BROOKLYN, N. Y.

THE ACTIVE CONSTITUENTS OF ERGOT: A PHARMACOLOGICAL AND CHEMICAL STUDY.*[1]

BY MARVIN R. THOMPSON.[2]

During 1929–1930, the writer published a series of ten reports embracing a review of the literature on Ergot and also the results obtained in certain pharmacological and chemical studies on Ergot and its more important pharmaceutical preparations. These reports presented evidence, in confirmation of a rather wide-spread unanimity of opinion, showing, among other things, that:

1. The amino-bases of Ergot (histamine, tyramine, cholines, etc.) could contribute little or nothing to the valuable therapeutic activity of the drug or any of its preparations.

2. The valuable therapeutic activity resided wholly in the "total specific alkaloidal fraction."

3. Of the four then known alkaloids, ergotinine and ergotaminine were comparatively inert, while both ergotoxine and ergotamine were indistinguishable in exhibiting intense activity by pharmacologic methods. Because of this great pharmacological activity, it was concluded that practically the full therapeutic activity of Ergot must reside in ergotoxine and/or ergotamine.

4. Aqueous Extracts of Ergot were practically worthless because they were invariably deficient in ergotoxine or ergotamine, and in addition were improperly standardized or not standardized at all.

5. Fluidextract of Ergot, U. S. P., or similar alcoholic or hydroalcoholic preparations, contained alkaloidal activity in satisfactory amounts and hence, such extracts were concluded to be superior to aqueous types of extracts.

6. Either ergotoxine or ergotamine was completely representative of the valuable pharmacological activity of Ergot, and, therefore, either of these alkaloids should be complete therapeutic substitutes for Ergot or its crude extracts.

Since publishing the above-mentioned reports, the author has continued to experimentally investigate certain phases of the ergot problem, largely because some of the most important conclusions regarding the activity and active principles of ergot have been based, by all workers in the field, upon experimental evidence of a much too indirect type, as for example, results obtained from experiments upon isolated uteri taken from non-pregnant and virgin animals. Such matters as absorption, changes in the uterus caused by pregnancy and the different stages of the œstrus cycle, and inherent differences in susceptibility between different animals, had been neglected by all pharmacologists up to 1930.

* Abstracted from a dissertation submitted to the Board of University Studies of the Johns Hopkins University, in conformity with the requirements for the degree of Doctor of Philosophy; reported in part before the Scientific Section of the annual convention of the AMERICAN PHARMACEUTICAL ASSOCIATION in Toronto, August 25, 1932, and in part at the annual meeting of the same body on May 10, 1934, at Washington, D. C.

[1] From the Department of Chemical Hygiene, School of Hygiene and Public Health, The John Hopkins University.

[2] Emerson Professor of Pharmacology, School of Pharmacy, University of Maryland; Consultant Pharmacologist, Food and Drug Administration, U. S. Department of Agriculture.

Of the conclusions drawn up to this time, the most important one requiring further confirmation in the way of more direct experimental evidence had to do with the active principles. Ergotoxine and ergotamine salts were being offered to the medical profession as full and complete substitutes for ergot or its crude pharmacopœial extracts. If these pure alkaloids were completely representative of ergot activity, there would be little justification for the continued use of the officially recognized crude extracts.

Earlier unpublished observations caused the writer to conduct a series of comparative experiments, using available salts of ergotoxine and ergotamine, and various crude extracts, for the purpose of determining whether or not the oxytocic effects of the purified alkaloids were actually the same as the effects produced by the crude extracts when administered to intact pregnant cats.

THE METHOD.

The afore-mentioned observations resulted in the selection of a method involving the use of pregnant cats because it was believed that of all experimental animals available for a large number of comparative experiments, the cat was the nearest possible approach to human conditions. For the adopted technique, anesthesia was necessary. Various barbituric acid derivatives such as nembutal, dial, amytal, phanodorn and phenobarbital, as well as ether, chloroform, chloretone and avertin have been used, but since all of these anesthetics were observed to depress uterine activity if the anesthesia became deep, and since pregnant cats vary considerably in their individual susceptibility to anesthetics, it was found distinctly advantageous to employ anesthetics which permit of intravenous administration so that the very cautious injection of divided doses could be carried out in such a manner that a relatively light anesthesia could be insured. The sodium salts of the shorter acting members of the barbituric acid derivatives were, therefore, used for most of the experiments. The use of inhalation anesthetics was abandoned in the beginning because they required an objectionable amount of constant attention, and also because respiratory irritation often caused decidedly objectionable coughing or sneezing during the maintenance of light anesthesia. A tracheal cannula was used to further avoid such disturbances. A somewhat heavier anesthesia, effected with ether, was usually employed during the operative procedure, but thereafter a lighter level of hypnosis was maintained.

Following anesthesia, the pregnant uterus was exposed, and the movements recorded upon the kymograph by the use of the movable arms of a myocardiograph, tying the arms into opposite ends of one of the more longitudinally contractile segments of one horn of the uterus. The animal was not immersed in a constant temperature bath, but a large electric warming pad was employed, and the small area of exposed uterus was constantly irrigated with Locke-Ringer solution at 37.5° C., the liquid dropping upon cotton partially covering the exposed part of the uterus. The entire procedure involves no hemorrhage.

A normal tracing was taken for approximately one-half hour or more in all instances before administering the ergot preparations. In the interpretation of the tracings reproduced in the illustrations of results obtained by this method, the relative magnitude of the recorded uterine responses cannot serve to show the actual relative intensity of the uterine activity produced by the different ergot preparations. The comparisons must be made with reference to presence or absence of response and *the rapidity of the onset of the response only*. The *magnitude* of the contractions in the different tracings is dependent, not alone upon the *intensity* of response induced by the ergot, but upon the contractility of the particular area of the uterus included between the two attached arms of the recording apparatus. Different uteri and different parts of the same pregnant uterus vary greatly in contractile power, and it was quite impossible to accurately judge the contractility at the time of attaching the recording apparatus. Some of the uteri showing the lowest magnitude of recorded response were observed to show a much greater actual response than other uteri which responded feebly but recorded enormous excursions of the writing lever on the kymograph. This could readily be noted simply by visual observations of the exposed uterus.

Using this technique, it is fully realized that the anesthesia and operative procedure results

in an "abnormal animal." The studies involved were, however, of a purely comparative nature, with the surgical procedure and anesthesia operating as a more or less cancellable constant. Nevertheless, to make this study as critical as possible, the effects of the various types of ergot preparations and chemical fractions or principles upon the strictly normal animal would obviously add to the significance of the results.

Accordingly, use was made of an observation that representative extracts of ergot cause abortion in pregnant cats with phenomenal regularity, regardless of the stage of pregnancy. The young are invariably born dead, not during the violent uterine activity caused by ergot, but following this activity by 6 to 24 hours. In the very terminal stages of pregnancy the young are occasionally born alive. This test is obviously not of an accurately quantitative nature, but considerable importance has been attached to it as indicating ergot "activity" or "inactivity" in the fundamental studies which follow.

COMPARISON OF THE ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS, AQUEOUS EXTRACTS AND AVAILABLE SALTS OF ERGOTOXINE AND ERGOTAMINE.

1. Pharmaceutical.—Suitable portions of 24 different lots of crude ergot, all assayed and found to be of U. S. P. potency or higher, were mixed together to yield a total of approximately 5 Kg. This material was ground and de-fatted by the U. S. P. method. An acid hydro-alcoholic fluidextract was prepared from 1 Kg. by the fractional percolation method described elsewhere by the writer (1), a method involving the use of no heat whatever. Percolation was continued until the combined percolates, when assayed by the modified (2) Broom-Clark method, exhibited an alkaloidal potency equivalent to 0.5 mg. of ergotoxine ethanesulphonate per cc. The volume of product was 3.790 liters. This product, designated as Fluidextract No. 360, was stored in the refrigerator at 0° to 5° C., small portions being removed for use as needed.

Another 1-Kg. portion of the same powder was converted into an Aqueous Extract exactly as above, except that water alone constituted the menstruum. Percolation was carried to the same volume as for the Fluidextract No. 360, *i. e.*, 3.790 liters. This product was designated as Aqueous Extract No. 361, and was stored in the same refrigerator as the Fluidextract No. 360. When assayed by the same method as the fluidextract, a potency equivalent to 0.19 mg. of ergotoxine ethanesulphonate per cc. was revealed. It will be noted that this was considerably less than half of the alkaloidal potency contained in the hydro-alcoholic fluidextract. *But it should also be noted that one cannot simply assume that aqueous extracts are alkaloid-free, even though they invariably contain less alkaloid than the hydro-alcoholic extracts.*

Both of the above extracts were also assayed by the U. S. P. Cockscomb method and by the colorimetric method of Smith (3). The writer, after several years of experience, holds little faith in the routine accuracy of the Cockscomb method unless an impossible number of birds are used. It may be stated, however, that both extracts gave satisfactory cockscomb reactions in agreement with the potency revealed by the more accurate Rabbit Uterus method. As to the colorimetric method, increased experience causes the writer to doubt the accuracy of the values originally obtained for the above extracts. Both extracts gave the color reactions, however, and the fluidextract, as by the other methods, proved to possess a higher alkaloid content than the aqueous extract. The colorimetric values were higher than the physiological values for both extracts. Studies relating to the color reaction and its quantitative value will be reported later. It is important

to point out that all three of these methods, which are believed to measure only alkaloidal activity, showed the presence of significant amounts of ergot alkaloids, even in the aqueous extract.

2. *Pharmacological.*—A number of experiments, recording the effects of oral doses of various ergot extracts as well as salts of ergotoxine and ergotamine upon the uterus of lightly anesthetized pregnant cats, showed beyond doubt that both aqueous and hydro-alcoholic extracts, as well as the commercially available salts of ergotoxine and ergotamine, were decidedly active in increasing the tonus and rhythmicity of the uterus. It was equally apparent that, viewing the results of four or more experiments upon each type of preparation, that the crude extracts, both aqueous and hydro-alcoholic, produced a uterine response which differed from that produced by either of the commercial alkaloidal salts. The chief difference was in the rapidity of the onset of response. Both of the above-described crude extracts acted much more promptly, usually well within ten minutes, and apparently more intensely than calculated equivalents of the ergotoxine and ergotamine salts. The effects of the alkaloidal salts developed only after 30 to 60 minutes. In these earlier experiments, the extracts were administered undiluted, the alkaloidal salts in 1:1000 dilution, all in 1- to 2-cc. oral doses by stomach tube.

Viewing all experiments, however, disturbing discrepancies were occasionally observed, particularly with respect to the promptness of action. The effects of the crude extracts usually developed within ten minutes, while the effects of the ethanesulphonate and phosphate of ergotoxine and the methanesulphonate and tartrate of ergotamine usually developed only after 30 to 60 minutes. But occasionally the effects of the crude extracts would be delayed until within the range of the time of action of the alkaloidal salts.

ABSORPTION FROM STOMACH AND INTESTINE.

Realizing that anesthesia has a depressant effect upon gastro-intestinal function, it was thought possible that the active principles of ergot might not be absorbed from the stomach, and that the passage of the small dose volume into the intestine was delayed, thus accounting for these discrepancies in the rapidity of onset of action. This posibility was then investigated.

In a series of 9 experiments upon pregnant cats, following the usual technique, pyloric ligations were performed before oral administration of the ergot preparation by stomach tube. In not one instance did the characteristic effects upon the uterus develop, even though ridiculously enormous doses of crude extracts were given (up to 10 cc. of the extracts) within two hours. Upon the removal of the ligature, after approximately two hours, an effect would manifest itself promptly, especially if a dose of 10 or 20 cc. of water were administered. A representative experiment is illustrated by the tracing in Fig. 1.

It appears quite obvious, therefore, that none of the active principles of ergot whether alkaloids or unknown substances, are absorbed to any significant extent from the stomach of the cat. Since the uterine activity could be made to manifest itself in no uncertain manner after removal of the pyloric ligature, it follows that absorption takes place from the small intestine. This was further confirmed in one experiment by leaving the pyloric ligature in place while a dose of 1 cc. of Fluidextract No. 360 was injected into the lumen of the small intestine by means

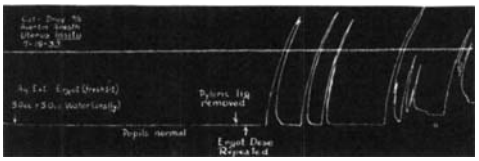

Fig. 1.—Cat: late pregnancy: uterus *in situ*. Time in minutes. Illustrating the lack of absorption of Ergot from the stomach and promptness of response of crude extracts following intestinal absorption. Pylorus ligated prior to administration of first oral dose of an Aqueous Extract known to be active. Note absence of oxytocic activity during two hours until ligature was removed, after which activity promptly developed. Hydro-alcoholic extracts in the same or even larger doses, likewise failed to show activity until the ligature was removed.

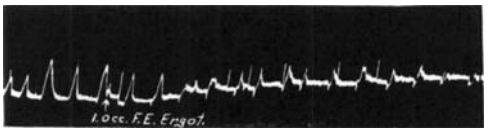

Fig. 2.—Cat: late pregnancy: uterus *in situ*. Time: Same as Fig. 5. The prompt (within ten minutes) response following the oral administration of 1.0 cc. of F. E. Ergot No. 360 plus water. The influence of respiratory movement is evident in this tracing. Magnitude of recorded contractions is low because of inclusion of only very small area of uterus between the two arms of the apparatus.

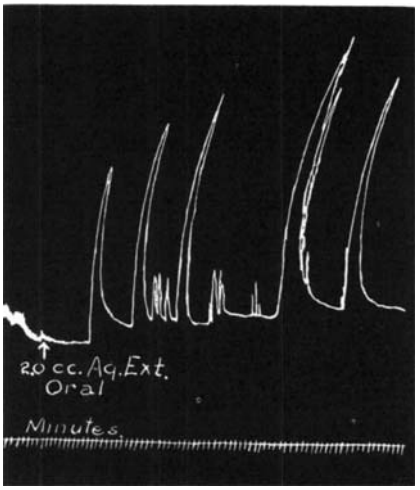

Fig. 3.—Cat: late pregnancy: uterus *in situ*. Prompt response following oral administration of 2.0 cc. of Aqueous Extract of Ergot with water.

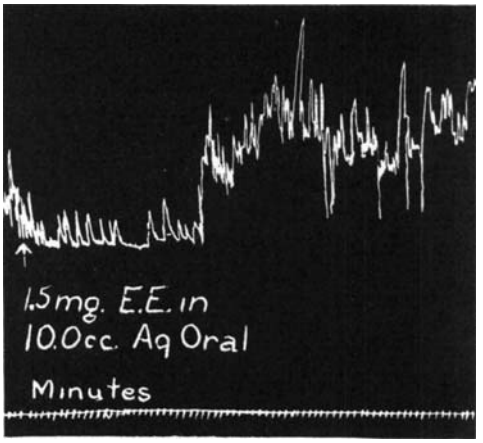

Fig. 4.—Cat: late pregnancy: uterus *in situ*. Delayed response following oral administration of large dose (1.5 mg.) of ergotoxine ethanesulphonate in 20 cc. water.

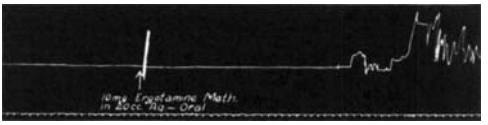

Fig. 5.—Cat: late pregnancy: uterus *in situ*. Time in minutes. The delayed uterine response following the oral administration of a large dose (1.0 mg.) of ergotamine methanesulphonate. After approximately one-half hour, however, pronounced activity is evident.

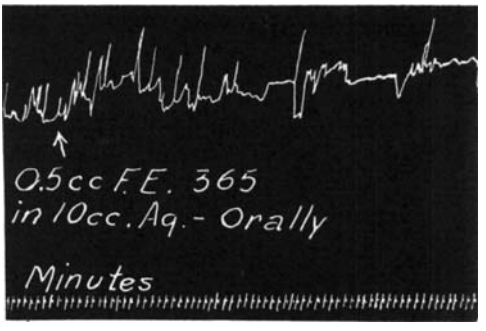

Fig. 6.—Cat: late pregnancy: uterus *in situ*. Prompt response following oral administration of 0.5 cc. of de-alcoholized F. E. No. 365 with water.

of a hypodermic needle. Uterine response resulted in $4^1/_2$ minutes, showing that intestinal absorption is quite rapid. Four similar experiments were performed using ergotoxine ethanesulphonate and ergotamine tartrate instead of a crude extract (two experiments upon each salt). No uterine effects were noted in any case within two hours while the pyloric ligation remained, even though the oral dose was 5 mg. in each case. Upon removal of the ligature, and administration of 20 cc. of water, uterine response developed only after 30 to 60 minutes, again revealing a great difference between these alkaloidal salts and the crude extracts. The effects produced by ergotoxine and ergotamine were indistinguishable by this method of study.

Because the comparative rapidity of onset of uterine action itself, following ergot administration into the gastro-intestinal tract, was the chief criterion to be used in distinguishing between the different constituents and preparations of ergot, it was necessary to use a procedure which would avoid the extremely variable delay in the passage of the dose from the stomach to the intestine. It was found that this variable could be satisfactorily removed by either of two ways. The drug could be injected directly into the lumen of the intestine, or the dose could be washed into the stomach with 10 to 20 cc. of warm water. The volume of water appeared to cause a fairly prompt opening of the pylorus in most instances, thus providing for a prompt transfer of the oral dose to the intestine for undelayed absorption. The animals were fasted for at least 12 hours before use.

DOSAGE.

The dosage administered to the cats was purposely large in practically all experiments. The method obviously has little quantitative value, but these studies were designed to simply determine presence or absence of oxytocic activity for various constituents and preparations of ergot, particularly with respect to type and promptness of action. The actual doses are indicated in the tables and illustrations.

DIFFERENCE BETWEEN NON-PREGNANT, PREGNANT AND POST-PARTEM UTERUS REGARDING RESPONSIVENESS TO ERGOT.

The use of non-pregnant cats was abandoned in the beginning of this investigation because the uteri of such animals responded only feebly or not at all, regardless of the size of the oral dose of either aqueous or hydro-alcoholic extracts of ergot. As a consequence the studies embraced in this report have involved the use of pregnant cats exclusively.

The cats used were those in which pregnancy was so far advanced that it could be readily detected by simple observation of abdominal distension or by palpation. While a considerable individual variation in sensitivity to ergot was observed, thus making observations rather obscure, the writer is left with the impression, after observing the uterine effects of ergot upon more than 250 cats in various stages of pregnancy and at various intervals post-partem, that the sensitivity or irritability of the uterus toward ergot increases as the pregnancy proceeds toward the termination of the gestation period. Greatest sensitivity to orally administered ergot appeared to be immediately preceding, during and immediately after, labor. After

TABLE I.—RAPIDITY OF ONSET AND INTENSITY OF UTERINE EFFECTS FOLLOWING ORAL ADMINISTRATION OF VARIOUS ERGOT PREPARATIONS TO LIGHTLY ANESTHETIZED PREGNANT CATS.

Type of Preparation.	Alkaloid Content.[1] Per cent	No. of Experiments.	Oral Dose.[2]	Average Time Required (Approx.) for Onset of Definite Uterine Effect. Minutes	Intensity of Uterine Effect.
Fluidextract, U. S. P.	0.050	6	2.0 cc.	6	Marked
Fluidextract, U. S. P.	0.120	2	1.0 cc.	6	Marked
Fluidextract, U. S. P.[3]	0.075	2	2.0 cc.	8	Marked
Fluidextract, U. S. P.[3]	0.03	2	2.0 cc.	8	Marked
Fluidextract, U. S. P.[3]	0.015	2	2.0 cc	4	Marked
Aqueous Ext. (Liq.)	0.019	6	2.0 cc	4	Marked
Aqueous Ext. (Liq.)	0.012	2	2.0 cc.	6	Marked
Aqueous Ext. (Liq.)	0.010	2	2.0 cc.	8	Marked
Tablets Ergotin, 3 grain[3]	0.000	3	20 tablets dissolved in water	No effect	None
Ergotin (Bonjean)[3]	0.125	2	0.5 Gm. in water	6	Marked
Ergotin (Bonjean)[3]	0.000	4	4.0 Gm. in water	No effect	None
Ergotoxine ethanesulphonate solution[4]	0.100	12	1.0 cc.	36	Doubtful to marked
Ergotamine tartrate solution[4]	0.100	15	1.0 cc.	38	Doubtful to marked
Histamine acid phosphate solution[4]		3	10.0 mg.	None	None
Tyramine hydrochloride solution[4]		2	100.0 mg.	None	None
Acetyl choline solution[4]		2	50.0 mg.	None	None

[1] Colorimetric method of Smith, in terms of ergotoxine ethanesulphonate.
[2] Washed in with 10 to 20 cc. of warm water, by stomach tube.
[3] Manufacturers label, age unknown.
[4] Solutions freshly prepared W/V; not assayed.

labor, the sensitivity appears to decline rapidly. By keeping a large number of pregnant cats on hand during the spring of 1932, it was possible to use two cats so nearly at term that true labor promptly followed the oral administration of 0.5 cc. of a U. S. P. Fluidextract of Ergot. Most powerful tonic uterine contractions started within five minutes in both cases, true labor developing in 35 minutes and 85 minutes, respectively. The uterine contents could not be expelled, of course, because of the abdominal incision. The violent abdominal muscular spasms forced uterus and contents through the incision to the exterior, throwing the recording apparatus out of adjustment and consequently ruining the kymograph tracings. Two other cats were used immediately after normal delivery was completed. A 0.5-cc. dose of U. S. P. Fluidextract, in two minutes for one and four minutes for the other, induced tonic contractions bordering on complete tetany. Uteri of cats used after the first day post-partem showed progressively decreasing sensitivity to ergot. After a week post-partem, the uterine response was observed to be very feeble, as in the case of the non-gravid uterus (3 experiments, 1 cat 49 hours post-partem, 1 cat 86 hours post-partem and 1 cat 196 hours post-partem). These few experiments cannot establish this important point conclusively, but if this general impression is correct even in part, there is a possibility that puerperal human patients may also gradually lose uterine sensitivity to ergot as the length

of the post-partem period increases. This may have some significance in studies similar to those of Moir (4, 5) in which the human patients are used not during the period when the action of ergot is most necessary and possibly most effective, but six days post-partem. In the specific studies of Moir, however, the point may be disregarded as far as the relative activity of different ergot preparations is concerned because his patients were all used at approximately the same post-partem interval. His comparative clinical evaluation of the different types of ergot preparations should, therefore, be perfectly valid. However, the possibility that all ergot preparations would produce a somewhat more intense effect than he observed, if given immediately at the termination of the third stage (when ergot is especially indicated), is a distinct possibility and is worthy of appropriate study.

Fractionation of Ergot into Active and Inactive Components.

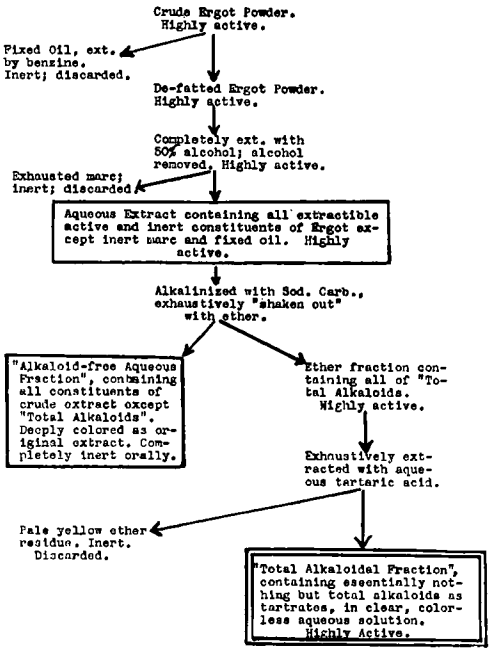

Fig. 7.—Diagram of chemical fractionation procedure.

WHAT ARE THE THERAPEUTICALLY ACTIVE PRINCIPLES OF ERGOT?

This investigation has furnished abundant confirmation of Moir's (4, 5) results showing that the commercially available salts of ergotoxine and ergotamine are possessed of oral activity, but that this activity falls far short of being wholly representative of the action of the drug itself or its crude extracts. It is evident, therefore, that there exists some unknown factor in the crude extracts. Typical tracings are shown in Figs. 2 for a Hydro-alcoholic Fluidextract, 3 for an Aqueous Extract, 4 for Ergotoxine Ethanesulphonate and 5 for Ergotamine Methanesulphonate. The tartrate and methanesulphonate of ergotamine exhibited the same indistinguishable type of delayed action. Table I presents a summary of the results of numerous experiments in which the uterine movements were recorded *in situ*. A highly important difference between the known alkaloids and the crude extracts becomes immediately evident upon examination of this table.

DISCUSSION OF TABLE I.

Considerable individual variation in these cats was noted. This was to be expected, because, entirely aside from a well-known individual variation in cats, it was obviously impossible to secure numbers of cats in the same stage of pregnancy. All were in advanced stages, however, because hair was well developed in the young in all cases (determined after sacrificing animals when experiment was terminated). Uterine effects of varying degrees of intensity were obtained from all preparations except the 3-grain ergotin tablets and one specimen labeled Ergotin (Bonjean). As observed by Moir on human patients, the salts of ergotoxine and ergotamine were decidedly less effective than the crude extracts, both as to promptness and intensity of effects, the alkaloids requiring 25 minutes or more, the extracts less than 10 minutes to produce their respective effects.

A significant difference between hydro-alcoholic and aqueous extracts was not revealed by this method in spite of the fact that the colorimetric method showed a higher alkaloidal content in the hydro-alcoholic extracts. It follows, therefore, that the aqueous extracts exhibited an activity entirely out of proportion to the alkaloid content, as observed by Moir in humans.

One decidedly interesting bit of information is furnished by these results. The only two preparations which proved totally inactive, even in ridiculously high doses, *were likewise the only two preparations for which a color reaction could not be obtained,* regardless of how much of each preparation was used for the color test. This point was critically investigated because the only two preparations failing to exhibit activity were those which actually proved to be completely alkaloid-free. It is very important to note that ergot preparations failing to give the color reaction, when the amount of preparation specified by Smith or by the B. P. Modification is used, actually need not be alkaloid-free. Good color reactions are obtained by this method only when the alkaloidal content of the preparation at least approximately approaches the level specified by the U. S. P. or the B. P. To prove actual freedom from alkaloids, approximately four or more times the usual amount should be used, the ether extract should be concentrated to less than the usual volume, and the acid aqueous extraction should likewise be reduced to about one-fourth the usual volume. Then when the reagent is added, and no color develops, one can conclude that the preparation is actually alkaloid-free for research purposes. These facts were kept in mind in obtaining the alkaloid values of Table I, the colorimetric readings being taken only when the intensity of color for standard and unknown was brought within plus or minus 20% of each other by using appropriate amounts of the preparations to be tested.

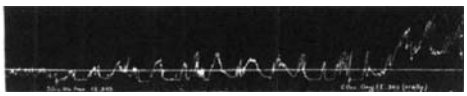

Fig. 8.—Cat: late pregnancy: uterus *in situ.* Showing lack of significant activity of "Alkaloid-free" F. E. No. 365 in very large oral dose (5.0 cc. with water). Note also the prompt and intense effect following the 2.0 cc. oral dose of the original F. E. No. 365 (from which the "Alkaloid-free F. E." was prepared), given over three hours later. Time in minutes.

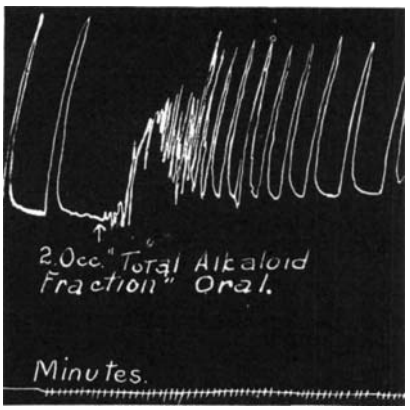

Fig. 9.—Cat: late pregnancy: uterus *in situ.* The prompt response following the oral administration of 2.0 cc. of "Total Alkaloidal Fraction" in 10 cc. water, prepared from F. E. No. 365.

Histamine, tyramine and acetyl choline did not contribute to the valuable activity of ergot preparations, as was concluded by the writer (6) in 1930, and by Moir (5) in 1932.

THE EFFECT OF ERGOT PREPARATIONS UPON NORMAL PREGNANT CATS.

Every ergot preparation included in Table I caused abortion within 36 hours in at least one pregnant cat, when given orally by stomach tube in large but non-lethal doses, *except histamine, tyramine, acetyl-choline and the two preparations which proved to be alkaloid-free.* The latter two preparations were without effect even in relatively enormous doses by either oral, subcutaneous or intramuscular administration, but those cats which failed to abort were subsequently (after three days or more) caused to lose their young by the administration of one of the other crude extracts or one of the salts of ergotamine or ergotoxine. Large necrotic abscesses were invariably produced by the subcutaneous administration of the larger doses of the crude extracts. By this method, therefore, it has been determined that the available salts of ergotoxine and ergotamine are decidedly active orally or parenterally. Likewise, hydro-alcoholic and aqueous extracts were decidedly active orally or parenterally unless they were alkaloid-free as determined colorimetrically. This method does not permit of a critical comparison between the salts of ergotoxine and ergotamine and the active crude extracts, because all induced abortion in an apparently similar manner.

Ten mg. of histamine acid phosphate, 50 mg. of tyramine hydrochloride or 20 mg. of acetyl choline, given individually or all in the same dose, by stomach tube, were ineffective in causing abortion (5 experiments).

The results of the investigation up to this point completely agreed with Moir's conclusion that the available salts of ergotoxine and ergotamine are not carriers of the full oxytocic activity of ergot. But inasmuch as it was possible to demonstrate the presence of alkaloids by their color reaction in every specimen which had proved active on either anesthetized or unanesthetized pregnant cats, it appeared quite probable that either the activity was completely due to the "total alkaloids" in some form or other, or else that the new unidentified non-alkaloidal "Moir principle" had disappeared with the alkaloids in the two inactive preparations.

THE DETERMINATION OF THE EXISTENCE AND CHEMICAL NATURE OF A NEW HIGHLY IMPORTANT SUBSTANCE IN ERGOT.

Extracts of ergot were prepared by using alcohol, ether, chloroform, acetone and other organic solvents. All preparations, administered after removal of the solvent, were active in varying degrees upon unanesthetized and anesthetized pregnant cats. The activity invariably proved to be of the "prompt new type" rather than the "delayed ergotoxine or ergotamine type." A 50% hydro-alcoholic menstruum proved to be decidedly the more efficient extraction menstruum. This information was of little aid, however, and considerable effort was then expended in isolating various pigments and other constituents for test. These efforts were apparently in the wrong direction since none of the fairly pure pigments or other non-alkaloidal constituents carried significant activity. The details of these unfruitful experiments will not be presented, because later work in a new direction completely ruled out the numerous pigments or colored constituents of ergot as carriers of oxytocic activity.

Observations up to this point appeared to justify the conclusion that a 50% hydro-alcoholic menstruum is capable of extracting every trace of active material from ergot if percolation is carried far enough. Such an extract, of course, contains much more inactive than active material, but it perhaps would provide the best starting point for the purpose involved. Accordingly, 1 Kg. of the defatted ergot powder described earlier in this report was subjected to exhaustive percolation with 50% alcohol by the fractional percolation method. Percolation was discontinued when the percolate could no longer be made to give the Smith color reaction. The

combined percolates were concentrated, without heat, under high vacuum to 1000 cc. The material was in contact with nothing but glass-ware at any time. The resulting deeply colored material was acid to litmus, and was essentially aqueous because all but traces of the alcohol had been removed during concentration.

This material was highly active as shown by its induction of abortion in three pregnant cats following 0.5-cc. oral dosage. It possessed the characteristic "prompt" type of oral activity as shown by three experiments upon lightly anesthetized cats, an example of which is illustrated in the tracing of Fig. 6. By the epinephrine-inhibition isolated rabbit uterus method, a total alkaloidal equivalent of 2.87 mg. ergotoxine ethanesulphonate per cc. was determined. This material was designated as Fluidextract No. 365.

It was reasoned that if the belief of Moir and Dale (5) that the total alkaloidal fraction did not carry the full activity of the drug were correct, the above fluidextract should show a great deal of activity after complete chemical removal of the alkaloidal fraction.

Complete removal of the "total alkaloids" was accomplished by slightly alkalinizing (to litmus) 500 cc. of the Fluidextract No. 365 by cautious admixture with finely powdered sodium carbonate, and exhaustively "shaking out" with many portions of ether, until the deeply colored aqueous fraction could no longer be made to yield the Smith color reaction, thus showing complete freedom from alkaloid. The deeply colored aqueous fraction was subjected to vacuum to remove residual ether. This alkaloid-free aqueous material, 500 cc. in volume, was designated as the "Alkaloid-Free Fraction."

The combined ether portions were concentrated to a volume of 500 cc., under vacuum without the aid of heat. All operations were carried out in the dark. The 500-cc. ethereal extract was then exhaustively extracted in a separatory funnel with many small portions of 1% aqueous tartaric acid solution until the Smith color reaction could no longer be obtained when final portions of the tartaric acid extract were reduced to one-fourth the volume and the aldehyde reagent added. The ethereal portion which had thus been completely exhausted of alkaloids, was placed under vacuum to remove the ether and the small amount of residue was incorporated in the "Alkaloid-Free Fraction."

The acid aqueous solution of the total alkaloids was reduced to a volume of 500 cc. by high vacuum, filtered and the small amount of precipitate (color negative) was incorporated in the "Alkaloid-Free Fraction." At this point the solution of total alkaloids still carried a pale yellow color. It was, therefore, purified by again alkalinizing with sodium carbonate and exhaustively shaking out with ether. The aqueous residue, after removal of the dissolved ether, proved completely inert on the uterus of both the anesthetized and unanesthetized pregnant cat in two divided oral doses totalling 50 cc., and was therefore discarded. The ethereal solution of the total alkaloids after reducing the volume to 500 cc. *in vacuo*, was washed several times with 0.5% sodium carbonate solution to remove most of the remaining pale yellow color. The sodium carbonate washings were unable to yield the color reaction and were completely inert, after neutralizing with HCl, upon oral administration to two pregnant cats in doses totaling 50 cc. These washings were therefore discarded. The washed ethereal solution of the total alkaloids was then again exhaustively extracted with small, divided portions of 1% aqueous tartaric

acid solution until all of the "total alkaloids" were contained in the acid aqueous solution (using the colorimetric test as the guide). The ether was again removed from the alkaloid-free ether fraction *in vacuo*, and the residue incorporated with the "Alkaloid-Free Fraction."

The purified aqueous tartaric acid solution was freed from dissolved ether and concentrated to 500 cc. *in vacuo* without heat, and filtered rapidly in the dark. The resulting product was absolutely clear and colorless. It should have contained essentially nothing but a solution of the "total alkaloids" of ergot in the form of their tartrates. This aqueous solution of the "total alkaloids" was designated as the "Total Alkaloid Fraction."

The diagram of Fig. 7 illustrates precisely what was done by the above chemical procedure to obtain the "Alkaloid-Free Fraction," containing essentially all of the extractives of ergot except the "total alkaloids," and the "Total Alkaloidal Fraction" which contained essentially none of the extractives of ergot except the "total alkaloids" in the form of their corresponding tartrates.

THE PHARMACOLOGIC ACTIVITY OF THE "ALKALOID-FREE FRACTION."

(*a*) *On the Cockscomb.*—Doses as high as five times the "threshold dose," or up to 3.0 cc. per Kg., failed to produce a cyanotic reaction in the combs of a series of ten cockerels meeting U. S. P. X specifications (as given under "Fluidextract of Ergot"). The "Alkaloid-Free Fraction" was, therefore, concluded to be inert by this method of test.

(*b*) *On the Isolated Uterus of the Virgin Guinea Pig.*—Activity equivalent to 0.15 mg. of histamine per cc. was determined by this procedure. The quality of activity was similar to that of histamine and was of a similarly transitory nature. The activity was entirely similar to that described earlier by the writer (7, 8).

(*c*) *On the Carotid Blood Pressure of the Anesthetized Dog.*—By intravenous administration, a transitory depressor effect similar to that produced by histamine was observed. The character of the tracings was essentially identical with those reproduced in an earlier publication (9). The amount of histamine-like activity was found to be, within the limits of experimental error, the same as observed on the isolated guinea-pig uterus.

(*d*) *By the Epinephrine-Inhibition Isolated Rabbit Uterus Method* (*2*).—No activity could be demonstrated by this method.

(*e*) *On the Uterus in Situ of the Anesthetized Pregnant Cat by Oral Administration.*—Using the same technique described earlier in this report, no uterine activity was observed in four experiments, even after a total of 50 cc. of the "Alkaloid-Free Fraction,' representing 50 Gm. of the original ergot, was administered to each cat during two and one-half hours. Each experiment was terminated after four hours or more by administering orally a dose of 1.0 cc. of the original unfractionated fluidextract. This produced significant uterine activity within ten minutes in each case, showing that failure of the "Alkaloid-Free Fraction" to induce activity was not due to unresponsive uteri. Illustrated by Fig. 8.

(*f*) *On Unanesthetized Normal Pregnant Cats.*—Oral doses up to 30 cc. failed to interrupt pregnancy in eleven cats. Two of the cats littered within four days after medication, but it is believed that the delivery was not brought on by the medication, as all of the young were born alive and fully developed. In abortion induced by large doses of ergot, the young are born dead unless the pregnancy is in the terminal stages. The cats appeared to remain free from any other symptoms following even the largest oral doses.

Subcutaneous doses of ten to fifteen cc. failed to interrupt pregnancy in eight pregnant cats. In the course of a week or ten days, enormous necrotic abscesses invariably developed around the site of injection. These abscesses healed with extreme difficulty, requiring the passage of many weeks before spontaneous healing was complete. Large scars remained after the healing.

It is concluded from the above pharmacologic studies on the "Alkaloid-Free Fraction" that no significant valuable uterine activity of any kind was contained

in this fraction. The pharmacodynamic activity observed upon the isolated guinea-pig uterus and upon the blood pressure of dogs is of a histamine-like character, is inactive orally, and contributes nothing of a desirable nature to the characteristic action of ergot.

This eliminates all of the deeply colored constituents as well as a large amount of other non-alkaloidal components of ergot as carriers of the activity of this drug. The fact that this fraction is completely inert upon the uterus of the pregnant cat, while still containing constituents capable of causing severe irritation in small doses and enormous slow-healing abscesses from large doses by parenteral injection, is an important observation. Complete exclusion of these constituents from ergot preparations intended for hypodermic use is very necessary. It is believed that the abscess formation is probably due simply to the non-dializable or non-absorbable nature of the material, thus creating an effect similar to that caused by any irritant, unabsorbable body inserted under the skin or into the muscle tissue.

THE PHARMACOLOGIC ACTIVITY OF THE "TOTAL ALKALOIDAL FRACTION."

(*a*) *On the Cockscomb.*—Following essentially the U. S. P. procedure in applying this method, using ergotoxine ethanesulphonate as the standard, this fraction was intensely active in causing cyanosis of the combs of ten cockerels. An activity corresponding to the equivalent of not less than 2.0 mg., and not more than 3.0 mg. of ergotoxine ethanesulphonate per cc. was determined.

(*b*) *On the Isolated Uterus of the Virgin Guinea Pig.*—This fraction was definitely active by this method. Doses of 0.02 cc. to 0.2 cc. in 100-cc. tissue chambers induced strong contractions. It was impossible to assign any definite value in terms of a standard because repeated dosage to the same uterine strips produced progressively decreasing responses, showing that the drug gradually induced a paralysis and that this effect could not be washed out with sufficient ease to permit of any accurate estimation. The action was similar to that described for ergotoxine, ergotamine and crude extracts in a previous report (7).

(*c*) *On the Carotid Blood Pressure of Anesthetized Dogs and Cats.*—A definite and prolonged pressor response was obtained in both dogs and cats by intravenous administration of 0.01 to 0.06 cc. per Kg. body weight. The response was indistinguishable from that illustrated in Figs. X and X-*a*, or as described for the "specific alkaloids" in an earlier report (9). Repeated dosage invariably led to a progressively decreasing response until the well-known "epinephrine-reversal" effect could be produced.

Oral doses of 1.0 cc., diluted to 20 cc. with warm water, failed to cause a significant change in the blood pressure of a cat. The pressor action, therefore, appears to be dependent upon the sudden appearance of relatively large amounts of the alkaloids in the circulation.

(*d*) *By the Epinephrine-Inhibition Isolated Rabbit Uterus Method* (*2*).—The potency by this method was found to be equivalent to 2.56 mg. of ergotoxine ethanesulphonate per cc. It will be noted that this value agrees fairly well with the value obtained, by the same method, for the original Fluidextract No. 365, from which this "Total Alkaloidal Fraction" was prepared. The difference in the two values is barely significant, and shows that the loss in alkaloidal activity sustained in the chemical fractionation procedure was not great.

(*e*) *On the Uterus, in Situ, of the Anesthetized Pregnant Cat by Oral Administration.*—After 31 experiments, following the technique already described, the writer is convinced that this "Total Alkaloidal Fraction," as well as three others prepared similarly, contained all of the significant characteristic uterine activity of ergot. The uterine effects usually developed well within ten minutes, differing from salts of ergotoxine or ergotamine in this respect. Figure 9 illustrates the promptness of action following oral administration of the "Total Alkaloidal Fraction." The promptness in action was indistinguishable from that observed following the administration of the original Fluidextract No. 365, from which this fraction was obtained. The promptness of action does, however, clearly show that the "total alkaloids" include a very important member in addition to ergotoxine or ergotamine.

(*f*) *On Unanesthetized Normal Pregnant Cats.*—The "Total Alkaloidal Fraction," in oral doses of 0.5 to 2.0 cc., interrupted pregnancy within 24 hours, in twelve cats in various stages of pregnancy. Subcutaneous or intramuscular doses of 0.25 cc. (4 cats by each injection route) likewise proved effective in terminating pregnancy. The young in all cases were born dead. Death of the cat occasionally resulted after abortion in experiments of this type, whether the preparation given was the "Total Alkaloidal Fraction," salts of ergotoxine or ergotamine, or any one of the active aqueous or hydro-alcoholic extracts. This was to be expected since most of the doses administered throughout this work were large.

The foregoing experiments upon the "Alkaloid-Free Fraction," and the "Total Alkaloidal Fraction," again demonstrate, as the writer previously concluded (7), that the isolated guinea-pig uterus method, as it is usually applied, is wholly unreliable as a means of evaluating the significant activity of ergot preparations, measuring chiefly the extremely variable and totally useless histamine type of activity rather than the alkaloidal activity.

GENERAL ANALYSIS OF RESULTS.

Moir's observations upon human patients to the effect that, given orally, both aqueous and hydro-alcoholic extracts of ergot produce a much more prompt and more effective uterine action than the available salts of ergotoxine or ergotamine, have been repeatedly confirmed in this study upon pregnant cats. It follows, therefore, that the crude extracts owe their superior activity either (*a*) to an entirely new unidentified substance as concluded by Moir, or (*b*) to a naturally existing unknown ergotoxine or ergotamine compound or combination differing from the available salts or ergotoxine and ergotamine, and which is more readily absorbed from the intestinal tract than the manufactured alkaloidal salts. The writer feels that "(*b*)" had to be considered as a possibility in spite of the fact that Moir believed his active aqueous extracts to be practically alkaloid-free, because evidence has been presented in this as well as in an earlier report (8) showing that such extracts, although containing a deficiency of alkaloids, are usually not actually alkaloid-free and that they may contain sufficient alkaloids to account for definite activity when administered to humans in large doses as used by Moir.

A further analysis of the present results, however, rather definitely rules out "(*b*)" as a possibility, because of the repeatedly demonstrated fact that the "Total Alkaloidal Fraction" contained every trace of the characteristic activity of the original quantitative de-alcoholized hydro-alcoholic extract. Specifically, this "Total Alkaloidal Fraction" could consist of essentially nothing but an acid solution of the "total specific alkaloids," and these obviously in the form of their corresponding tartrates. This should preclude the possibility that the crude extracts owed their superior oral activity to a "mysterious" salt, or combination, of the two important well-known alkaloids ergotoxine or ergotamine, in spite of the fact that neither of these alkaloids have ever been proved to exist as such, or as simple salts, in crude extracts or ergot itself.

This leaves only "(*a*)" as a possibility. Their possibility developed into a virtual certainty when it was observed that salts of ergotoxine and ergotamine fell far short of being completely representative of the "total alkaloidal" activity of ergot, and also that the only other known important pharmacodynamically active constituents, namely, histamine, tyramine and acetyl choline, were not responsible for any of the characteristic oral activity of the drug. It may be concluded,

therefore, that Moir's and Dale's belief that a new highly important unidentified substance exists in ergot and its crude extracts (both aqueous and alcoholic), is confirmed by these studies. Quite contrary to their apparent belief, however, this new substance is shown to behave characteristically as an alkaloid, since the studies here reported show the presence of this new substance in the completely colorless "Total Alkaloidal Fraction," while the "Alkaloid-Free Fraction" proved to be devoid of any oral or subcutaneous activity of any significant type. The writer, therefore, believes that this new substance must be classified chemically as a new member of the "total specific alkaloids" of ergot, and that the activity of aqueous extracts, observed by Moir in human patients, is to be ascribed to "residual alkaloid."

(To be continued.)

ANTIDOTES. I. GENERAL PLAN.*

BY JAMES C. MUNCH, AND F. E. GARLOUGH.[1]

Men from the earliest times have endeavored to find ways of relieving pain or injury to themselves by means of spirits, charms, mysticism, and later by use of herbs and animals. In these efforts they found some herbs and later extracts that were either beneficial or deadly. It was also observed that the animals were either beneficially or injuriously affected by certain plants or animals which they ate, as is reflected in the names—cowbane, sowbane and wolfbane which are plants poisonous to cattle, swine and wolves.

The fatal effects resulting from the bites of animals brought about the first real effort to find antidotes to poisons, though some earlier knowledge had been gained of the counter-action of one drug upon another. In Homer's "Iliad" describing the Trojan War and the adventures of ancient heroes (*Ca.* 1000 B.C.) many references are made to the wounds of the warriers being rubbed with a bitter, pain-assuaging root. Homer mentions 250 such cases. The earliest reference to a plant which was specifically believed to be an antidote for poison is found in Homer's "Odyssey;" "Moli" or "Molu" which is apparently a species of Allium (*Allium moly*). In this case Circe's potion was a form-transforming drug, the effects of which moly supposedly counteracted.

Among the earlier writers on antidotes was the Greek, Nicander of Colophon (185–135 B.C.), the Court Physician to Attalus, King of Pergamum, who made studies of poisons on condemned criminals, a common practice at that period. His first work, "Theriaca," which was written in Greek verse, had to do with the poisons of animal bite sand treatments of them. His second poem, "Alexipharmaca," tells of antidotes to poisons. In these he mentions twenty-two poisons, including aconite, cantharides, opium and conium. His chief antidotes include warm oil, mallow and linseed tea to excite vomiting.

The development of antidotes to poisons was greatly stimulated when, about the time of Mithridates (first century B.C.), poisons began to be extensively used

* Scientific Section, Madison meeting, 1933.

[1] Bureau of Biological Survey, Glen Olden, Pa., and Denver, Colo.