SCIENTIFIC SECTION

THE ACTIVE CONSTITUENTS OF ERGOT.

A PHARMACOLOGICAL AND CHEMICAL STUDY.

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THE CHEMICAL ISOLATION OF A HITHERTO UNIDENTIFIED MEMBER OF THE "TOTAL SPECIFIC ALKALOIDS" OF ERGOT.

It was believed, after many unsuccessful attempts in various directions, that the most fruitful procedure to follow in attempting to isolate the new promptly acting alkaloid would be to obtain the total alkaloids in as pure a condition as possible, and then to remove all of the alkaloids having the slow ergotoxine or ergotamine type of activity, leaving the promptly acting new alkaloidal principle or principles uncontaminated as far as the known alkaloids are concerned. This proved to be a successful procedure.

Since the "Total Alkaloidal Fraction" was completely clear and colorless, and since pharmacologic tests had shown this material to contain essentially every trace of the significant activity of the drug, this activity residing wholly in the "total alkaloids" as tartrates, this solution provided an excellent starting point. Accordingly 1 Kg. of defatted Ergot was worked up to this point in the previously described manner. This colorless acid aqueous solution was then again alkalinized and exhaustively shaken out with many small portions of ether. The Smith colorimetric test and pregnant cats were extensively used to follow the extractions to prevent loss of alkaloids. As usual, all operations were carried out in the dark to prevent oxidation of these labile substances. The ethereal solution was then taken to complete dryness *in vacuo*, first over $CaCl_2$, then over P_2O_5, without heat.

This dry amorphous total alkaloidal residue could be purified and freed from most of the known inert (ergotinine, ergotaminine) or "orally slow acting" alkaloid (ergotoxine and/or ergotamine (?), etc.) in a number of ways because of the observation that the new alkaloidal substance, either in the form of the free base or its corresponding salts such as sulphate, phosphate, hydrochloride, tartrate, etc., was found to be far more soluble in all of the common organic solvents (methyl and ethyl alcohol, ether, chloroform, acetone, benzene, carbon bisulphide, carbon tetrachloride) than any of the four well-known alkaloids. Especially remarkable was the observation that the new alkaloid was somewhat soluble in water even as the free base, in marked contrast to ergotoxine or ergotamine. Separation of the "orally slow acting" ergotoxine or ergotamine type of alkaloid from the "orally quick acting" new alkaloid could therefore be accomplished either by: (*a*) Dissolving the above total alkaloidal residue in anhydrous ether, converting the alkaloids to their corresponding sulphates causing a precipitation of most of the "orally slow-acting" alkaloids which settle out in an over-night period, leaving the "orally quick-acting" new alkaloid in solution, or, (*b*) Dissolving the total alkaloidal residue in acetone, and cautiously adding water until precipitation of the ergotoxine or ergotamine type of alkaloid was complete, with subsequent removal by filtration, leaving the new alkaloid in solution.

By either procedure, the filtrate contained the new "orally quick-acting" alkaloidal substance, which was obtained in an amorphous condition upon removal of the solvent *in vacuo*. In two trial lots, this amorphous alkaloid was purified further by redissolving the material in 0.5% aqueous tartaric acid and taking advantage of the observation that partial saturation with sodium bicarbonate causes precipitation of the new alkaloid from concentrated solutions. The substance

was then collected upon a filter in the dark. and promptly dried in a desiccator *in vacuo*. Working with 1 and 2 Kg. quantities of ergot, the yield was 176 mg. and 221 mg. of alkaloid per Kg. of original ergot, respectively, indicating that the percentage yield is increased when larger lots of the drug are used.

CHEMICAL NATURE OF THE NEW ALKALOIDAL SUBSTANCE.

The new substance has not been obtained in a chemically pure completely crystalline condition. Quantitative data, therefore, will not be given at this time.[1] The approximately quantitative information which follows is presented solely because of the important bearing the results of this report have, *first*, upon the clinical observations of Moir pointing to a new important non-alkaloidal constituent in Ergot, and *second*, upon the suitability of currently used methods of assay and standardization for U. S. P., B. P. and other Pharmacopœial preparations of this drug.

The new alkaloidal substance contains all of the elements contained in ergotoxine (10), ergotamine (11), ergotinine (12), and ergotaminine (11), namely, C, H, O and N. The proportions of these elements likewise have been found to roughly approximate those of the known alkaloids in a single analysis of the (impure ?) material. The figures are withheld pending further analyses upon material of known purity, but it may be stated that this analysis indicates that the molecule of the new alkaloid is definitely smaller than that of either ergotoxine, ergotinine, ergotamine or ergotaminine.

It is insoluble in petroleum ether, but definitely more soluble in water, ethanol, ether, benzene, methanol, the chlor-ethylenes and the chlor-methanes, than the known alkaloids. It appears in representative, but variable, amounts in all freshly prepared liquid extracts such as Fluidextract of Ergot, U. S. P., and Liquid Extract of Ergot, B. P. It likewise appears in powdered and pilular extracts, such as "Ergotin" or Aqueous Extract of Ergot, N. F. V, in extremely variable amounts, depending largely upon the amount of heat employed in the manufacturing

TABLE II.—ALKALOIDS OF ERGOT.

No.	Alkaloid.	Discoverer and Year Reported.	Composition (Supposed).	Oxytocic Activity Following Oral Administration.	Van Urk (19) or Smith (3) Color Reaction.	Cockscomb and Isolated Rabbit Uterine (2) Reaction.
1	Ergotinine (12)	Tanret (1875)	$C_{35}H_{39}O_6N_5$ (Barger)	Negligible	Positive	Negligible
2	Ergotoxine (10)	Barger and Carr (1907)	$C_{35}H_{41}O_6N_5$	Delayed but somewhat active on cat and human	Positive	Powerful
3	Ergotamine (11)	Stoll (1920)	$C_{33}H_{35}O_5N_5$	Delayed but somewhat active on cat and human	Positive	Powerful
4	Ergotaminine (11)	Stoll (1920)	$C_{33}H_{35}O_5N_5$	Negligible	Positive	Negligible
5	Pseudo-ergotinine (13)	Smith and Timmis (1931)	$C_{35}H_{41}O_6N_5$ (assigned with reservation)	Unknown (probably inert)	Positive	Negligible (?)
6	Sensibamine (14)	Wolf (1931)	$C_{31}H_{37}O_5N_5$	Unknown	Positive	Powerful
7	Ergoclavin (15)	Küssner (1934)	$C_{31}H_{37}O_5N_5 \cdot H_2O$	Unknown	Positive	Powerful
8	"X alkaloid"	Thompson (1934)	C H O N (mixture (?)) formula not yet assigned	Promptly and highly active on cats and humans*	Positive	Powerful

* See appended note at end of article.

[1] This amorphous "X alkaloid" has very recently yielded a crystalline product. Pharmacological and chemical studies upon these crystals are being pursued. Precise qualitative and quantitative data may, therefore, be presented in a later report.

process. Boiling temperatures gradually destroy the alkaloid in aqueous medium, but the destruction is usually far from complete after many hours, especially if partial vacuum is coincidently employed to affect concentration of the extract. Concentration of extracts in metallic vessels, with heat *in vacuo* or otherwise, markedly accelerated the destruction of this active substance as well as the other alkaloids.

The new alkaloid is much more stable than the ergotoxine type of alkaloid in crude extracts (either liquid or solid). Oxidation, spontaneous or chemically induced, causes the substance to become yellow, and finally a dark brown. This oxidation is attended by a corresponding decline in oxytocic activity.

Tested colorimetrically by the Smith method, or as modified by the 1932 B. P., the blue color was readily obtained. Of five different lots prepared to date, the intensity of the color reaction ranged from approximately 30% (for the less purified material) up to 60% (for the more purified material) of that produced by equivalent concentrations of ergotoxine ethanesulphate.

It is of interest at this point to consider the possible identity of the new alkaloid, and its possible relationship to the four well-known alkaloids, as well as some alkaloidal substances which have been obtained and described by others since 1931. These alkaloids, exclusive of substances known to be degradation products of the alkaloids, with their discoverers, etc., may be tabulated as in Table II.

It will be observed from Table II that all of the known alkaloids are closely related chemically. They can be distinguished from one another, however, by differences in solubility, optical rotation, melting points (especially their crystalline salts which generally melt more sharply than either the crystalline or amorphous bases themselves), ease of crystallization, crystallizing medium, and character of crystals, but the very practically important and readily utilizable method of distinguishing between them is to be found in the promptness of their pharmacological and clinical activity following oral administration.

Alkaloids Nos. 1 and 4 may be at once dismissed as unimportant because they are practically inert even upon the isolated uterus. No. 5 probably falls in the same class, because of its extremely close relationship to the inactive ergotinine (13). They are pharmacologically inert, thereby differing in the main essential from Nos. 2, 3, 6, 7 and 8. Nos. 2, 3, 6, 7 and 8 all appear to be pharmacologically active when tested upon the isolated rabbit uterus or other hitherto widely employed pharmacologic methods. Nos. 2 and 3, however, differ greatly from No. 8 in that the oxytocic response following oral administration to pregnant cats is much more prompt and effective for No. 8 than for Nos. 2 and 3. As to Nos. 6 and 7, nothing can be said from the standpoint of promptness or effectiveness when given orally, since they were not available for this study, nor has any such information been made available by others. Regarding No. 6, Barger (16) stated that Stoll had challenged the claim that this was a new alkaloid, because upon mere recrystallization ergotamine was obtained. (Note chemical similarity between 6 and 7.) As to the relationship existing between Nos. 7 and 8, it can only be stated that they are both highly active upon the isolated rabbit uterus, they appear to be similar as to solubility, both being water-soluble, but whether No. 7 exhibits the prompt oral activity of No. 8 is unknown. None of No. 7 was available for this study because of the fact that the report by Küssner (15) was obtained just prior to the sending of this report to press.

PHARMACOLOGIC ACTION OF THE NEW ALKALOID.

(*a*) *Upon the Cockscomb.*—The U. S. P. Cockscomb method measures this alkaloid along with the ergotoxine or ergotamine present in ergot preparations. 0.2 to 0.4 mg. per Kg. consistently produced definite cyanosis of the combs.

(*b*) *By the Epinephrine-Inhibition Isolated Rabbit Uterus Method* (*2*).—The purest of the five lots prepared to date acted in a manner qualitatively indistinguishable from ergotoxine or ergotamine. Quantitatively, the new substance appeared to be slightly more than half as potent as ergotoxine, in its possibly contaminated amorphous state.

(*c*) *Upon Carotid Blood Pressure of Anesthetized Cats and Dogs.*—Injected intravenously, a pressor action was demonstrated upon both cats and dogs, the effects upon the two species being similar. An example of this activity is shown in Fig. 10. The pressor effect was also similar to that of either ergotoxine or ergotamine by the intravenous route.

The "vasomotor reversal" was readily produced in two cats, as illustrated in Fig. 10-*a*. Repeated dosage produced gradually diminishing pressor response until doses similar to the first effective dose would no longer produce an effect.

Oral doses up to 1 mg. produced no significant change in carotid blood pressure of cats during one hour.

(*d*) *Upon the Isolated Uterus of the Guinea Pig.*—These effects, using immature virgin uteri, were likewise indistinguishable from those of the "Total Alkaloidal Fraction," already described.

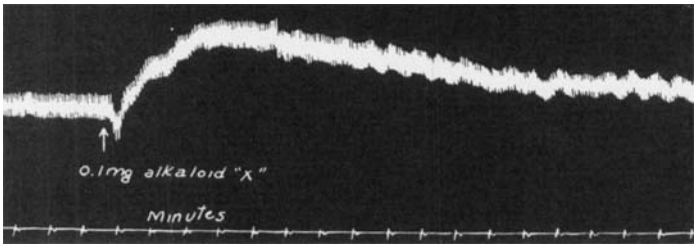

Fig. 10.—Male cat: 2.9 Kg.: Dial-urethane anesthesia: carotid blood pressure. The pressor response following the intravenous administration of 0.1 mg. of "X alkaloid."

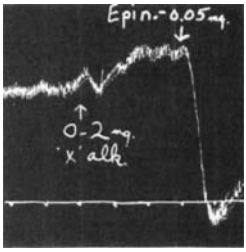

Fig. 10-*a*.—Male cat: 3.1 Kg.: Dial-urethane anesthesia; carotid blood pressure, illustrating the "vasomotor-reversal" induced by the "X alkaloid." Minutes.

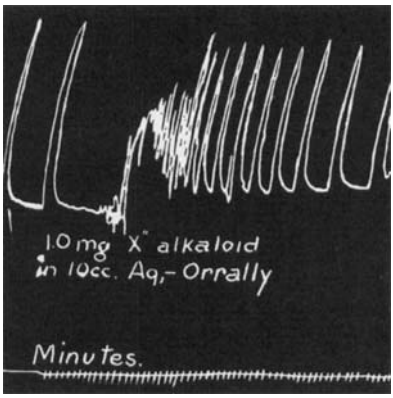

Fig. 11.—Cat: late pregnancy, uterus *in situ*. The prompt response following the oral administration of 1.0 mg. "X alkaloid," with 10 cc. of water.

Following the method previously described by the author (7), using mature uteri, histamine response was effectively inhibited in a manner similar to that produced by ergotoxine, ergotamine or the "Total Alkaloidal Fraction."

(*e*) *Upon Normal Unanesthetized Pregnant Cats.*—Either oral (1.0 mg.) or subcutaneous (0.5 mg.) doses were consistently effective in terminating pregnancy in a total of 21 cats in various stages of pregnancy. The young were invariably dead at birth. Death of two cats resulted after abortion, following a subcutaneous dose of 1.0 mg. to each, but this dose was not always fatal.

(*f*) *Upon the Uterus of the Lightly Anesthetized Pregnant Cat.*—This was the only pharmacologic method which clearly revealed a difference between the new alkaloid and ergotoxine or ergotamine.

Recording the movements of the uterus of the pregnant cat *in situ*, in the usual manner, oral doses of 1.0 to 2.0 mg. of the new alkaloid in 10 to 20 cc. of water have been observed to produce a uterine response usually well within five minutes, rarely beyond ten minutes, in 17 experiments.

Corresponding doses of ergotoxine or ergotamine, as shown earlier in this report, are active in causing a response, but never in less than twenty minutes (usually thirty minutes or more). An example of the prompt response is illustrated in Fig. 11. The activity is of long duration. Experiments have been observed up to four hours at which time evidence of activity had not ceased to be apparent.

SUMMARY AND CORRELATION OF RESULTS.

The uterine activity of various "type" extracts of ergot and commercially available salts of ergotoxine and ergotamine has been studied by observing the effects following oral, subcutaneous and intramuscular administration to anesthetized and unanesthetized cats in the later stages of pregnancy.

The method involving the recording of the uterine movements of the pregnant cat *in situ* provided a means of pharmacologically demonstrating an important difference between ergotoxine or ergotamine, and crude aqueous or hydroalcoholic extracts of ergot, confirming Moir's clinical observations on this point. These studies have also confirmed Moir's conclusion that there exists in ergot a highly important new "as yet unidentified substance" in ergot.

Contrary to Moir's apparent belief, however, the present studies have shown that all of the activity obtained from his various ergot preparations, could have been obtained from no constituents of ergot other than one or more of the "total alkaloids." Even in the case of his "Aqueous Extracts," which are admittedly deficient in ergot alkaloids, the present studies have demonstrated that all of the significant activity shown by these preparations could have been due to no substances other than "residual alkaloid."[1]

[1] In a recent lecture (November 14, 1933) on ergot in London by Professor G. Barger (16), the lecturer discussed, among other things, the clinical observations of Moir pointing to a new important non-alkaloidal principle in ergot. "The lecturer stated that this was also his own opinion up to about three months ago, when he thought that that was the situation. One U. S. worker, Marvin Thompson, who has published some accurate estimations of Ergot varieties, had raised doubts in his mind as to whether the action was not, after all, due to some residual alkaloid. He would like to say very little on the subject, and he put forward the suggestion with some hesitation." The discussion provoked by Prof. Barger's lecture was also published by the same *Journal* (16). This discussion was very interesting to the present writer, inasmuch as the comments were made by a number of workers who have been most active in this field, including Dr. Moir, Sir Henry Dale and Professor Burn. It is quite obvious, from the comments made by these workers, that they had little faith in the contention of "the American worker" that the activity observed by Moir from Aqueous Extracts was due to "residual alkaloid." Professor Burn stated that "it was difficult to see how 'residual alkaloid' could produce an effect in a patient, either quicker or as quickly as any other physiological effect." Dr. Moir stated, in part, that "The point was that an 'inactive' preparation proved to be active," obviously implying that an alkaloid-free preparation was clinically active. Sir Henry Dale stated, among other things, that "With regard to his (Professor Barger's) statements, referring to work in America, which suggested that the action was due to residual alkaloid, he did not believe it. He thought that the amount of residual alkaloid was quite trivial."

Replying to these comments, the present writer refers the reader to the evidence of this report, including the appended note. It was this evidence which was discussed with Professor Barger during his visit in Baltimore, and which caused him to make mention of the matter in his lecture. In the studies reported by Moir, it is believed that the prompt and impressive activity he obtained from all of his extracts was due to ergot alkaloids. Although he assumed his aqueous extracts to be essentially alkaloid-free, assays of numerous such extracts by the present writer have usually shown them to contain an alkaloidal content of 0.05 to 0.20 mg.

A chemical procedure for completely separating ergot into its "total alkaloidal" and "non-alkaloidal" fractions has been described. The colorless "Total Alkaloidal Fraction" has been found to contain all of the significant oxytocic activity of the drug, while all of the remaining constituents of ergot contained in the "Alkaloid-free" fraction were found to be completely devoid of any valuable oxytocic activity.

The "Total Alkaloidal Fraction" was observed to exhibit oxytocic activity in a manner superior to that of ergotoxine or ergotamine, proving that the "Total Alkaloidal Fraction" contained at least one hitherto unknown alkaloid.

A chemical procedure has been described for fractionating the "total alkaloids" by removing the ergotoxine, ergotamine, ergotinine and ergotaminine, leaving the new alkaloidal substance in solution. A method for the purification and isolation of the new substance in amorphous condition likewise has been presented. This substance, as it was obtained for these studies, was not proved to be a pure chemical entity, but was highly active.

The new alkaloid was found to differ pharmacologically from the hitherto known active alkaloids mainly in the promptness of its oxytocic action, especially following oral administration. The action of the new alkaloid usually developed in one to ten minutes, while the action of ergotoxine and ergotamine developed in twenty-five to sixty minutes, following oral administration of large doses to pregnant cats. Hitherto available methods (blood pressure, cockscomb, isolated tissues, etc.) failed to significantly distinguish qualitatively between ergotoxine or ergotamine and the new alkaloid. This is undoubtedly the reason for the hitherto well-established, although erroneous, opinion among pharmacologists that either ergotoxine or ergotamine were completely representative of the total activity of the drug or its extracts.

The results make it appear that the chief important differences between the new "X alkaloid" and the well-known ergotoxine or ergotamine are that the former is more soluble and more rapidly absorbable than either of the latter alkaloids. Little, if any difference is evident upon blood pressure following *intravenous* administration, or when compared upon *isolated* smooth muscle. It should be emphasized, however, that while the two types of alkaloid act similarly upon *blood pressure* following intravenous administration, the activity upon the *uterus in situ*, by the same route, is by no means similar. The activity of the new alkaloid is definitely more prompt and pronounced than that of ergotoxine or ergotamine, although the difference is not as great as when both types of alkaloid are given orally. It would appear, therefore, that the effect of either upon blood pressure is almost immediately dependent upon the concentration in the blood stream, but that the uterine effect is produced only after some further absorption or diffusion from the blood stream, perhaps into the lymphatic circulation, before exerting an effect upon the uterine innervation.

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per cc. in terms of either ergotoxine or ergotamine. Since Moir administered doses of such extracts ranging from 4.0 to 16.0 cc., it should be observed that the alkaloidal equivalent of such doses would range somewhere between 0.2 and 3.2 mg. The present writer has found that the greater part of the total alkaloids of aqueous extracts consists of the new alkaloid instead of ergotoxine or ergotamine, because of the greater ease of extraction and greater stability of the new alkaloid. Interpretation of Moir's results in the light of the results of this study, shows that the oxytocic effects he obtained from even his aqueous extracts were certainly due to "residual alkaloid," and this consisting principally of the new alkaloid here described.

It has been demonstrated that the active principles of ergot (total alkaloids) are not absorbed from the stomach of the cat, and that absorption occurs only after passage through the pylorus into the intestine. It is believed that this is probably likewise true in humans because of the fact that the time for onset of action from a given active preparation is not absolutely constant for different patients (see appended note, also Moir's results). Administration of the oral dose with 10 to 20 cc. of water causes a much more consistently prompt response in cats, than when the same dose is given without water, presumably due to a more prompt opening of the pylorus stimulated by the larger bulk of fluid.

Aqueous and hydro-alcoholic extracts, when injected subcutaneously or intramuscularly, were observed to be intensely irritant. In the large doses, huge, slow-healing abscesses were produced. This is not an uncommon experience with clinicians. The severe irritation and abscesses have been shown to be caused, not by the valuable colorless (in solution) oxytocic constituents of ergot, but by the difficultly absorbable, deeply colored, inert fraction. The presence of these difficultly absorbable inert constituents in injectable ergot preparations should be condemned.

Hydro-alcoholic types of liquid extracts of ergot, when freshly and properly prepared, have been shown to contain all of the "total alkaloids" of the drug. They are, therefore, completely representative of the drug itself, as far as oxytocic activity is concerned. They are suitable for oral administration only, because they contain the irritant and difficultly absorbable inert constituents, and are, therefore, prone to cause pain and abscesses upon hypodermic injection.

Aqueous types of liquid extracts of ergot do not contain all of the "total alkaloids" of the drug. They have been found to contain practically all of the promptly acting new "X alkaloid," but only insignificant amounts of the slow acting ergotoxine or ergotamine type of alkaloid. Although the promptly acting "X alkaloid" is the more important because of its prompt action, the slow-acting ergotoxine or ergotamine type of alkaloid is, nevertheless, significantly active, undoubtedly adding considerably to the duration of effect. Such preparations, therefore, cannot be regarded as being completely representative of the drug. They are suitable for oral administration only, because they contain the irritant and difficultly absorbable inert constituents, and are, therefore, prone to cause pain and abscesses upon hypodermic injection.

The value of solid or pilular extracts (ergotins) for oral administration depends entirely upon the type of procedure used in their manufacture, particularly with respect to the amount of heat used. They can be prepared so as to be highly active, and if physiologically standardized, should be dependable. Most of such extracts available at the present time have had much of their alkaloidal activity destroyed by the excessive heat used in their manufacture (from the examination of 37 commercial samples, the manufacturing process of which was precisely known by the writer for 19 of the samples), and practically none of them is standardized. They owe the greater part of any activity they possess to the new "X alkaloid."

The currently available methods of assay and standardization have been studied with reference to their value in insuring activity in ergot preparations, and their relative merits discussed. The U. S. P. Cockscomb method, the Broom-Clark Rabbit Uterus method, the Thompson Histamine-Inhibition Guinea-Pig

Uterus method, and the Smith colorimetric method, with the various modifications of each, have been shown to measure the new alkaloid along with the ergotoxine or ergotamine type of activity, but none of these methods can readily serve to distinguish between the prompt-acting new alkaloid and the slow-acting ergotoxine or ergotamine type alkaloid as these alkaloids exist in official extracts. Inasmuch as the new alkaloid is extracted easier, and appears to be much more stable than the ergotoxine or ergotamine type alkaloid (as these alkaloids exist in Pharmacopœial ergot preparations), it is believed that the use of any one of the above methods can be applied in such a manner as to insure satisfactorily standardized amounts of activity in such extracts. The method should, therefore, be chosen from the standpoint of routine reliability and precision. From this standpoint, the author favors a modification of the Broom-Clark Rabbit Uterus method (2), but has become favorably impressed with the merits and possibilities of the Colorimetric method. These methods will receive more specific consideration in a separate communication.

All of the above methods require the use of a "standard of comparison." The writer has examined all of the only four lots of U. S. P. Standard Fluidextract ever prepared and distributed by the U. S. Department of Agriculture. Nos. 635, 636 and 2160 have been shown to be unstable in an earlier report (17). The last lot prepared and distributed, No. 2835, has been observed to lose not less than half of its original activity from the time it was first tested, in July 1932, until last tested in January 1934. This standard, therefore, fails in its purpose.

The author (18), in 1930, recommended that either ergotoxine ethanesulphonate[1] or ergotamine tartrate be adopted as the bioassay standard in place of the faulty "U. S. P. Standard Fluidextract." These two salts have now been employed by the writer for over five years in testing ergot preparations. Both have been found to be satisfactory from the standpoint of stability. Since the newly discovered alkaloid shows a type of activity which is measured by all of the assay methods enumerated above, being practically indistinguishable from ergotoxine or ergotamine by such methods, and since a preparation of the new alkaloid of proven stability cannot be available for at least several years, it is believed that the adoption of one of the alkaloidal salts previously recommended is a virtual necessity. Until more appropriate methods of assay are available, a perfectly stable salt of the new alkaloid would have no advantage over available ergotoxine or ergotamine salts as a bioassay standard of comparison. In the light of evidence to date, including Moir's observations upon humans, the observations set forth in this report, and the studies by Koff upon humans (see appended note), the relationship existing between quantitative bioassay results (U. S. P. or other methods measuring "total alkaloidal activity") may be illustrated by taking a properly prepared representative U. S. P. Fluidextract of Ergot as containing all of the therapeutically active principles of the drug. Arbitrarily assigning the value of 100% to both the clinical activity and the bioassay (cockscomb, etc.) activity, the new "X alkaloid" is present in such proportions as to account for 15 to 25% of the bioassay activity, but owing

[1] The ergotoxine ethanesulphonate used in these studies was very kindly supplied by Dr. Clifford S. Leonard, Burroughs Wellcome & Co. For the generosity in supplying the ergotamine tartrate and methanesulphonate here employed, the author is indebted to The Sandoz Chemical Works.

to the great clinical effectiveness of this alkaloid, it accounts for approximately 75% of the clinical activity. The slow and feebly acting (clinically) ergotoxine or ergotamine type of alkaloid in such a fluidextract accounts for 75 to 85% of the bioassay activity, but only 15 to 25% (approx.) of the clinical activity. The "X alkaloid" is extracted much more easily and is more stable than the ergotoxine type of alkaloid. Therefore, a fluidextract may assay as low as 15 to 20% of U. S. P. requirements and still retain the greater part of its clinical oxytocic activity.

CONCLUSIONS.

1. The pregnant cat has been found to be a suitable test subject upon which to study comparatively the oxytocic activity of various types of preparations and constituents of ergot.

2. A procedure, involving oral administration of the ergot preparations, has been described and used extensively for the above purpose. Such a procedure can be successfully employed in investigating the chemical source of the significant oxytocic activity of ergot.

3. Carefully prepared hydro-alcoholic extracts of ergot, such as Fluidextract of Ergot, U. S. P. (U. S. P. X or Interim Revision), or Liquid Extract of Ergot, 1932 B. P., contain all of the important active principles of the drug. Such preparations are rich in alkaloids and are remarkably prompt and effective upon the uterus following oral administration.

4. Aqueous extracts of ergot do not contain all of the important active principles of the drug. They are deficient in ergot alkaloids, but are never alkaloid-free unless they are many years old or the alkaloids have been destroyed by excessive heat in their manufacture. When carefully prepared, these extracts are remarkably prompt and effective upon the uterus following oral administration. This prompt and effective activity is entirely out of proportion to the ergotoxine or ergotamine equivalents of such preparations.

5. Ergotoxine and ergotamine are indistinguishable in producing a much delayed and erratic action following oral administration. The activity of these alkaloids is, therefore, far from being completely representative of the drug itself or its crude extracts, as formerly supposed.

6. A hitherto unknown, highly important, active principle exists in ergot.

7. Every trace of the significant oxytocic activity has been found to reside in the chemically purified "total alkaloids" of the drug, even in the so-called aqueous extracts, as shown by the prompt activity obtained from the "Total Alkaloidal Fraction" in contrast to the complete lack of significant activity in the "Alkaloid-Free Fraction."

8. Ergotoxine and ergotamine are not representative of the "total alkaloidal activity." The new active principle appears, therefore, to be another member of the specific alkaloids of ergot, since it followed the other alkaloids in the chemical procedure used in obtaining the "Total Alkaloidal Fraction."

9. The activity of aqueous extracts observed by Moir must have been due, contrary to his belief, to "residual alkaloid" consisting mainly of the new alkaloid described in this report. The alkaloidal deficiency of such extracts is due to the inefficiency of water in extracting the ergotoxine or ergotamine. Most of the more

stable new alkaloid is readily extracted by water and hence appears in fairly representative amounts in such extracts.

10. The new alkaloid has been isolated in a sufficiently pure amorphous condition to permit of certain pharmacological and chemical comparisons with the hitherto known alkaloids.

11. The new alkaloid is closely related to ergotoxine and ergotamine as is shown by similar chemical behavior and also as is shown by its similar pharmacological action when tested upon the isolated guinea-pig uterus, the isolated rabbit uterus, the cockscomb and the carotid blood pressure of cats or dogs. Its activity persists for hours, as does that of ergotoxine and ergotamine.

12. The new alkaloid differs from ergotamine and ergotoxine mainly by its much more soluble nature, and by its more prompt and powerful oxytocic action following oral administration. The greater solubility, together with the probability that the new alkaloid has a smaller molecule, compared with ergotoxine or ergotamine, undoubtedly accounts for the more prompt absorption and greater effectiveness of the new alkaloid.

13. All of the remarkable observations of Moir can be explained by the demonstration of the existence of the new alkaloid.

14. None of the active oxytocic principles of ergot (the specific alkaloids) are absorbed to any significant extent from the stomach of the cat, following oral administration.

15. All of the active oxytocic principles of ergot (the specific alkaloids) are absorbed with varying degrees of rapidity from the intestine of the cat following oral administration. The new alkaloid is promptly absorbed while ergotoxine and ergotamine are absorbed with great difficulty. This difference in absorption rate also manifests itself following subcutaneous or intramuscular injection.

16. Ordinary aqueous or hydro-alcoholic extracts of ergot are intensely irritant to the tissues following subcutaneous or intramuscular administration. Severe abscesses develop at the site of injection, especially following the larger doses.

17. The irritant and abscess-forming properties are not due to the important active principles (the specific alkaloids) of ergot. They are due to the otherwise pharmacologically inert extractives appearing in the liquid extracts.

18. The color of an ergot preparation is no indication of its value or activity. The purified, total active principles are colorless in solution.

19. Either ergotoxine ethanesulphonate or ergotamine tartrate constitutes the best available "standard" for comparison in the evaluation of ergot preparations by the currently accepted quantitative methods.

20. The Isolated Guinea-Pig Uterus method, as usually applied (as in testing Liquor Pituitarii, U. S. P.), is wholly unreliable as a means of insuring significant activity in ordinary aqueous or hydro-alcoholic extracts. It measures chiefly the worthless non-specific amine activity of such extracts.

21. Clinical activity in reasonably standardized amounts can be insured by requiring official liquid ergot extracts to contain a total specific alkaloidal activity, equivalent to approximately 0.05 per cent, in terms of either ergotoxine ethanesulphonate or ergotamine tartrate, when tested by the Cockscomb method, the Epinephrine-Inhibition Rabbit Uterus method or the Colorimetric method. This will provide for the presence of essentially all of the more important new alkaloid

present in the parent drug, plus varying but larger proportions of the less important ergotoxine or ergotamine. None of these methods can serve to differentiate between the new alkaloid, ergotoxine or ergotamine in crude extracts.

22. Solid or pilular extracts can be made to contain a satisfactory amount of activity by extracting properly and avoiding the use of excessive heat and exposure to oxygen in the process of concentration.

23. The non-specific amino-bases of ergot (histamine, tyramine, cholines, etc.) contribute nothing of a desirable nature to the characteristic oxytocic activity of the drug.

NOTE ADDED MAY 1, 1934.

Through the kindness of Dr. Arthur K. Koff, Woman's Clinic, The Johns Hopkins Hospital, some clinical confirmation of the pharmacological and chemical evidence of this report has been obtained. During the past two years, Dr. Koff has conducted over fifty experiments upon human patients, recording the oxytocic action of drugs in a manner essentially similar to that employed by Dr. Moir in England. After observing the clinical response of different ergot preparations, he has, among other things, confirmed Dr. Moir's observations that both the aqueous and alcoholic types of crude extracts, given orally, cause a remarkably prompt and intense uterine response, entirely out of proportion to their ergotoxine or ergotamine equivalent, and that oral doses of salts of ergotoxine and ergotamine produce only a much delayed and feeble response even in very large doses.

Upon being informed of the pharmacological and chemical results obtained by the author, Dr. Koff very kindly agreed to observe the activity of certain preparations upon human patients, recording the uterine contractions directly as in Moir's experiments. The author's "Alkaloid-Free Fraction," freshly prepared as described in the above report, except that 1 cc. represented 2 Gm. instead of 1 Gm. of ergot, administered orally to three patients, proved to be completely inert in doses up to 10 cc., representing 20 Gm. of the original ergot. The original fluidextract, from which the "Alkaloid-Free Fraction" was prepared, produced the characteristic prompt and pronounced effects of the crude extracts upon these same patients, in 4-cc. doses (representing 4 Gm. ergot), the effect developing within fifteen minutes in every case.

An oral dose of 4.0 cc. of "Total Alkaloidal Fraction," prepared as described in the above report, and representing 4.0 Gm. of the original ergot, administered to one patient, produced a pronounced effect well within fifteen minutes. The promptness of action was indistinguishable from that produced by the fluidextract from which both "fractions" were prepared. The effect was still pronounced after nine hours, observations being discontinued at this point.

The orally "slow-acting" ergotoxine or ergotamine type of alkaloid, separated in the usual described manner from the "Total Alkaloidal Fraction," proved to be devoid of the prompt type of activity following an oral dose of the alkaloid equivalent to 8 Gm. of the original ergot to one patient. No effect whatever was evident for more than an hour, after which a feeble effect, entirely similar to that produced by available salts of ergotoxine and ergotamine, was produced.

These clinical experiments are especially significant because of the fact that the difference between the delayed and erratic ergotoxine or ergotamine effect and the prompt action of crude extracts is so great and so consistent that it is quite unnecessary to carry out numerous experiments in order to show the difference. It will be noted that these experiments upon humans have confirmed the results obtained upon the pregnant cat in every case. The "Alkaloid-Free Fraction" was capable of producing no oxytocic effect upon cat or human. The "Total Alkaloidal Fraction" produced a prompt and intense oxytocic effect upon both cat and human. The ergotamine or ergotoxine type" of alkaloid removed from the "Total Alkaloidal Fraction" produced a much delayed feeble effect upon the human and the cat (huge doses to cats produce an intense effect, but it is always much slower in developing than in the case of the crude extracts or the "Total Alkaloidal Fraction"), entirely similar to that produced by commercially available salts of ergotoxine and ergotamine.

The details of these clinical experiments will be more completely described elsewhere by Dr. Koff.

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Full responsibility is assumed by the writer, however, for any errors in presentation, results or conclusions which may appear in this report.

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SOME PHARMACOLOGICAL AND BACTERICIDAL PROPERTIES OF UMBELLULONE.*[1,2]

BY MILES E. DRAKE[3] AND ERNST T. STUHR.

INTRODUCTION.

The literature on the oil of the California laurel, *Umbellularia californica* (Hook. and Arn.) Nutt., includes very little information on the pharmacology of the oil, or of the ketone (umbellulone) obtained from it. Likewise, no reference was

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[1] An abstract based upon a thesis by Miles E. Drake submitted to the faculty of the Graduate School of the Oregon State College, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

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