THE PHARMACOLOGY OF ERGOT: WITH SPECIAL REFERENCE TO BIOLOGICAL ASSAY AND STANDARDIZATION.

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

VI. A METHOD FOR THE PREPARATION OF A PURIFIED FLUID-EXTRACT OF ERGOT,* INCLUDING PHARMACOLOGICAL STUDIES.

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The majority of present-day pharmacologists agree that the therapeutic value of ergot or its preparations is wholly dependent upon the specific alkaloidal content. These alkaloids represent but a small portion of the extractable material occurring in the crude drug, although not all of this material is pharmacodynamically active. In addition to the specific alkaloids, the extractable material includes the coloring principle known as sclererythrin, 10 to 35% of a fixed oil, exceedingly variable proportions of a series of water-soluble amines (histamine, tyramine, etc.) and very appreciable amounts of inert proteinogenous substances.

The manufacture of a Fluidextract of Ergot by the process specified in the U. S. P., 10th Revision, has been found to result in a preparation which contains practically all of the alkaloids and amines which are present in the parent drug (42). In addition to these pharmaco-dynamically active principles, much of the inert extractive material is also contained in the finished product. Because of its alcohol and acid content, Fluidextract of Ergot is suitable only for oral administration.

Of all the various constituents associated with ergot, only the specific alkaloids (ergotamine and ergotoxine), and the non-specific amines (histamine and possibly tyramine) have been found to exist in sufficient amounts to exhibit significant pharmaco-dynamic activity from reasonable doses. The alkaloids are active by either oral or hypodermic administration, but histamine, to which practically all of the non-specific amine activity of ergot is due, shows activity only when injected intravenously or intramuscularly. It is destroyed in the gastro-intestinal tract when given orally (14). Mammoser, Albi and Boyd (46) observed no absorption of histamine when introduced into the duodenum and colon of anesthetized dogs in doses of 5 mg. per Kg. Even when administered hypodermically, histamine produces only a very transitory effect upon the uterus of experimental animals and it is believed that the doses required as a parturient would be so large as to be actually dangerous because of the transitory, but powerful depressant effect of histamine upon blood pressure. The fact that histamine is the most powerful of all of the constituents of ergot in stimulating contractions of *excised* or *isolated* smooth-muscle tissue is undoubtedly responsible for the belief among many of the earlier investigators that the observed clinical efficacy of ergot was due principally to this constituent. Reasons for the proven ineffectiveness of histamine by

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* Method developed at the Department of Pharmacology, College of Pharmacy, George Washington University; included in thesis submitted June 1, 1929; pharmacological studies carried out in the Pharmacological Laboratory of the Food, Drug and Insecticide Administration, U. S. Department of Agriculture.

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oral administration have already been cited. Its unsuccessful hypodermic use in parturition cannot be attributed to poor absorption but recent pharmacological studies indicate that other factors prevent significant activity, as follows:

(*a*) *The natural resistance or protective mechanism of the body against histamine.*

In a preceding article of this series (42), epinephrine was shown to inhibit or antagonize the effect of stimulating concentrations of histamine upon the isolated uterus of the rabbit, *i. e.*, epinephrine could be made to abolish the response of the isolated uterus to histamine. Perla and Marmorston-Gottesman (43) have described experiments which clearly demonstrated the protective influence afforded against histamine poisoning in suprarenalectomized rats by the subcutaneous administration of epinephrine. Weiss, Soma, Ellis and Robb (45) reported extensive experiments involving the continuous and uniform intravenous administration of histamine to man, in which they observed that the introduction of 0.02–0.04 mg. of histamine per minute of a solution 1 : 10,000 was destroyed almost as rapidly as it entered the blood stream, thereby affording excellent evidence of an epinephrine-histamine antagonism in humans, and concluded that epinephrine, in a ratio of 1 : 30 antagonizes the effect of histamine on the cutaneous and cranial vessels, and that this epinephrine-histamine antagonism varies for different functions affected by histamine.

Thus, the foregoing experimental evidence adequately explains the unsuccessful use of histamine as a parturient or oxytocic by either oral or hypodermic administration, and also accounts for the fleeting or transitory nature of the effects produced by even relatively large intramuscular or intravenous doses.

(*b*) *Ergot alkaloid-histamine antagonism.*

Parts II (38) and III (39) of this series of articles contained evidence of an ergot alkaloid-histamine antagonism. The contractions induced by histamine on the isolated guinea-pig uterus could be inhibited or totally abolished by the action of ergot alkaloids. Geiger (44) also demonstrated this ergot alkaloid-histamine antagonism in studies on isolated frog liver. Histamine, in a concentration of 1 : 50,000 was found to increase the glycogenolytic effect of isolated frog liver. By first treating the liver with ergotamine (1 : 10,000), the histamine effect was prevented.

Articles IV (41) and V (42) of this series contained conclusive evidence that it would be practically impossible to insure defined amounts of the amines in a Fluidextract of Ergot even if their inclusion with the alkaloids were desirable. First, in confirmation of earlier reports of other investigators, the amines were found to be non-specific for the drug, some samples being apparently devoid of histamine or tyramine, others containing quantities varying from traces to over 0.1% of active amines, the most important of which was histamine. Second, the active amines of ergot were found to be very unstable in the menstruum of the fluidextract, causing a disappearance of significant amine activity within a period of several months even in samples originally showing an unusually high amine content (see Part V, (42) of this series of articles).

One would therefore be led to conclude, after a consideration of available information, that the non-specific amines are not responsible for any of the desirable clinical effects of ergot when administered alone or in mixture with the specific alkaloids, by either oral or hypodermic administration.

Since *only* the specific alkaloids of ergot are responsible for the clinical value of ergot preparations, the exclusion of as *much* as possible of the remainder of the extractive material can be regarded only as advantageous. Although there seems to be no direct evidence to the effect that the non-specific amines are harmful in oral ergot preparations, it is highly probable that they are at least partly responsible for the transitory gastric disturbances (vomiting, nausea, etc.) occasionally observed following the administration of Fluidextract of Ergot because of the powerful local action of histamine upon the smooth muscle of the stomach. Certainly there is no possibility that the exclusion of non-specific amines would detract from the clinical value of a Fluidextract of Ergot, while it is quite possible that such procedure would at least partially obviate undesirable side actions and provide for a more rapid absorption of the alkaloids.

Entirely aside from clinical aspects, the pronounced pharmaco-dynamic activity of the non-specific amines has been found to be the cause of marked interference in attempts to assay or standardize ergot preparations by many of the currently used bio-assay methods. (See Parts II (38), III (39) and V (42) of this series of articles.) The exclusion of active amines from a preparation obviates this interference.

METHOD FOR THE EXCLUSION OF NON-SPECIFIC AMINES AND REDUCTION OF INERT EXTRACTIVES IN FLUIDEXTRACT OF ERGOT.

This method depends principally upon the complete solubility of the non-specific amines, and the relative insolubility of the specific alkaloids of ergot, in water.

Part IV (41) of this series of articles contained experimental evidence showing that adequate extraction of powdered, de-fatted ergot with water efficiently removed all of the non-specific amines but only insignificant amounts of the specific alkaloids; that the addition of small amounts of acid caused the removal of somewhat greater amounts of the alkaloids in addition to all of the amines; and that by mildly alkalinizing the aqueous menstruum, the amines could be extracted but practically none of the alkaloids appeared in the percolate. These results suggest that preliminary extraction of the de-fatted drug with water or an alkalinized aqueous menstruum could be made to remove the non-specific amines without extracting the specific alkaloids. The amine-free drug could then be subjected to the solvent action of a suitable menstruum for the extraction of the specific alkaloids.

In the examination of hundreds of samples of crude ergot, it was observed that practically all of them possessed a natural low acidity which varied with the different samples. Part of this acidity apparently is entirely independent of that resulting from the development of rancidity in the fixed oil occurring in ergot, since a varying degree of acidity was found to remain in the powdered drug even after being completely de-fatted. This natural acidity causes solution and subsequent loss of part of the active alkaloids when freeing the drug from amines by the preliminary extraction with water. A proper correction of this acidity can be made to effectively obviate the loss of appreciable amounts of alkaloids.

Since alkaloids are usually insoluble in alkalies, it would seem that any one of the commonly used alkalies added to the aqueous menstruum would prevent the loss by extraction of alkaloids. Unfortunately, complications arise which prevent such a simple solution of this problem. The active alkaloids of ergot exhibit amphoteric reactions, *i. e.*, they can be dissolved by the addition of either acids or slightly higher concentrations of alkalies. Therefore, to give the correct amount of a given alkali to be added to the aqueous menstruum for the removal of amines from all lots of ergot without causing a loss of alkaloids would be practically impossible, because the natural acidity of different lots of ergot is an extremely variable factor, making it very difficult to maintain the correct alkalinity of the aqueous menstruum.

This difficulty has been overcome by the use of a series of non-toxic reagents which exhibit neutral or only feebly alkaline reactions *per se*, but which are capable of entirely neutralizing

the natural acidity of ergot and maintaining only a very slight alkalinity in the amine-extracting aqueous menstruum. These reagents include sodium-bicarbonate; magnesium oxide, hydroxide and carbonate; calcium carbonate, etc. The efficiency of aqueous admixtures of these reagents in extracting the non-specific amines and excluding the specific alkaloids from the aqueous percolate has been studied by testing percolates for the presence of alkaloids with the paradimethylaminobenzaldehyde reagent of Van Urk (36), Tanret's test (see Dutch Pharmacopœia) and the nitrobenzaldehyde reagent (see *Analyst*, 1929, page 424). The results of these studies indicated that sodium bicarbonate is the most satisfactory of the alkaline reagents and that proper preliminary percolation of de-fatted ergot with water containing suitable proportions of sodium bicarbonate could be made to completely remove the non-specific amines and much of the inert material without extracting or losing appreciable amounts of the specific alkaloids.

Such strongly alkaline reagents as the carbonates or hydroxides of sodium, potassium and ammonium cannot be used without danger of losing appreciable amounts of the alkaloids because of the fact that either a very slight deficiency or excess of alkali provides for a removal and subsequent loss of alkaloids. Ammonia water was found to be the most satisfactory of this group, but requires very careful control. The use of the mildly alkaline insoluble reagents such as magnesium oxide, etc., is objectionable because the excess of the reagent cannot easily be removed from the drug. The excess causes a partial neutralization of the acid contained in the menstruum ultimately used to extract the alkaloids. This difficulty is not encountered with the use of sodium bicarbonate, since the excess of this reagent can be removed completely by washing the drug with water.

After the removal of the amines (histamine, etc.) and much of the inert material (coloring matter, proteinogenous substances, etc.) by percolation with alkalinized water, the drug can be subjected to percolation with an acid-hydro-alcoholic menstruum such as is used in the preparation of Fluidextract of Ergot, U. S. P. (24). This menstruum has been found to be suitable and efficient for extracting the alkaloids from ergot and in preparing a satisfactory product.

DETAILS OF METHOD FOR PREPARATION OF PURIFIED FLUIDEXTRACT OF ERGOT.

1. De-fatting the Drug.—Completely de-grease or de-fat 1000 grams of coarsely powdered ergot by the method described in the U. S. P., 10th Revision, and expose to a current of air until free from the odor of benzine.

2. The Aqueous Treatment for the Removal of Non-specific Amines and Inert Substances.—Mix the de-fatted drug with a sufficient amount of water containing 5% of sodium bicarbonate to render it evenly and distinctly moist, and to maintain it so during maceration for two to four hours in a tightly-covered container in a cold room. Prepare a glass or glass-lined percolator by placing, at the bottom, first a uniform layer of water-wetted cotton, then a layer of fine washed sand, then a close-fitting filter paper, and another layer of sand. Carefully pack the moistened drug loosely and uniformly on top of the filtering medium in the percolator, and add the sodium bicarbonate aqueous menstruum (5%), constantly maintaining a stratum of liquid above the drug, until the liquid begins to drop from the lower orifice of the percolator. Close the orifice, cover the percolator and allow to macerate for an over-night period (16 to 24 hours) *in a cold room*. Then allow the percolation to proceed at a slow rate, adding a menstruum consisting of pure water as required to maintain a stratum of liquid above the drug. Test portions of the percolate directly from the percolator from time to time for amines by the Isolated Guinea-Pig Uterus Method previously described (38). Continue the percolation with water until the percolate is found to be practically free from active amines. Discard this aqueous percolate. Then allow all of the liquid to drain completely. Carefully remove all but a 1- or 2-inch layer of the drug without disturbing the filtering medium of the percolator, and remove as much of the remaining water as possible by strong expression or centrifugation.

3. Extraction of the Ergot Alkaloids.—Transfer, in portions, the expressed drug to the percolator adding and mixing a sufficient amount of alcohol containing 2% hydrochloric acid U. S. P., after each portion, to render the drug uniformly saturated. When all of the drug has been thus returned to the percolator, add a sufficient amount of the acidified alcohol to maintain a slight stratum of liquid above the drug. Cover the percolator tightly and allow to macerate for 12 to 24 hours, or an over-night period. Then proceed with percolation at a slow rate, using a menstruum composed of 50% alcohol, 48% water and 2% hydrochloric acid, U. S. P. (see

Menstruum No. 1, Fluidextract of Ergot, U. S. P. (24)), until 750 cc. of percolate is obtained. Reserve this portion and continue percolation with diluted alcohol U. S. P. until the drug is exhausted of alkaloids. Concentrate the exhaust percolate at a moderate temperature *in vacuo* to a volume of 250 cc. and incorporate in the reserve portion. All operations must be carried out in a cold room to prevent decomposition of the unstable alkaloids, and to prevent fermentation during the preliminary aqueous extraction (fermentation causes a production of amines (41)). Assay the product biologically (see below) and adjust to the potency of U. S. P. Fluidextract of Ergot.

The finished product is of purplish red color, transparent, of characteristic odor and taste, and may be diluted with water without the development of more than a slight opalescence. It should be stored in amber-colored bottles in a refrigerator. Any sediment which collects on the bottom of containers may be rejected.

THE EFFICIENCY OF THE METHOD.

For the purpose of illustrating the efficiency of the method in excluding non-specific amines, etc., and providing for a preparation containing significant quantities of the active ergot alkaloids, a complete example involving a sample of ergot of predetermined composition is necessary.

Accordingly, a sample of crude ergot was selected which was known to contain a high non-specific amine content, and also a significant quantity of specific alkaloids. A portion of this sample was converted into a fluidextract by the U. S. P. process, and a similar portion was subjected to the process here described for the preparation of a Purified Fluidextract of Ergot. Each preparation was then carefully bio-assayed for non-specific amine content by the Isolated Guinea-Pig Uterus Method previously described (38), and for specific alkaloidal content by the Broom-Clark Isolated Rabbit Uterus Method (28), the results of which are given in Table VI.

TABLE VI.—A COMPARISON OF U. S. P. FLUIDEXTRACT OF ERGOT AND "PURIFIED FLUIDEXTRACT OF ERGOT."

Preparation.	Specific alkaloidal content in terms of ergotamine* base, per cent.	Non-specific amine content in terms of histamine,** per cent.	Total solids (47), per cent.
Fluidextract of Ergot, U. S. P. X	0.085	0.097	10.2
"Purified Fluidextract of Ergot"	0.075	None	2.75

* Ergotamine Tartrate (Sandoz Chemical Co.) containing 84.5% ergotamine base.
** Histamine (Pfanstiehl Chemical Co.).

Table VI is self-explanatory. The freshly prepared U. S. P. Fluidextract contained very appreciable quantities of amines in addition to the alkaloids, while the Purified or De-aminized Fluidextract contained none of the amines, but nearly as much alkaloidal activity as the U. S. P. preparation. A slight loss of alkaloidal activity cannot be avoided because of their lability in the presence of air, and the fact that, even though the alkaloids are not soluble in the alkaline aqueous menstruum, as light colloidal suspension results which causes a washing out and subsequent loss of a small portion of the alkaloids through the filter at the bottom of the percolator during the process of freeing the drug from non-specific amines. By observing a reasonable amount of care in the extraction technique, all traces of important non-specific amines may be removed without a loss of more than 20 per cent of the alkaloidal activity, even in those samples of

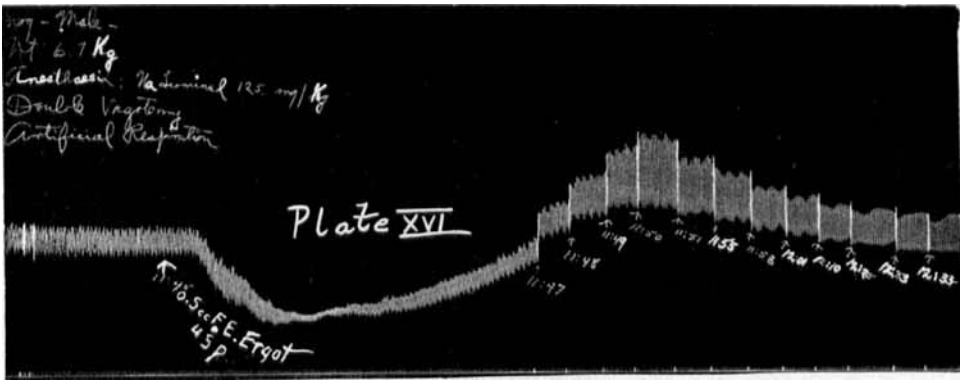

Plate XVI.—Showing the effect upon the carotid blood pressure of a dog produced by the intravenous administration of 0.5 cc. of Fluidextract of Ergot (Table VI), freshly prepared by the process of U. S. P. Note that the initial fall in pressure, presumably due to histamine, is followed by a perceptible rise in pressure, probably due to the alkaloids. Thus, both the non-specific amines and the specific alkaloids are shown to be present in significant amounts in this preparation.

crude ergot bearing an unusually high proportion of non-specific amines. Table VI also shows the relatively low proportion of total solids which appears in the Purified Fluidextract.

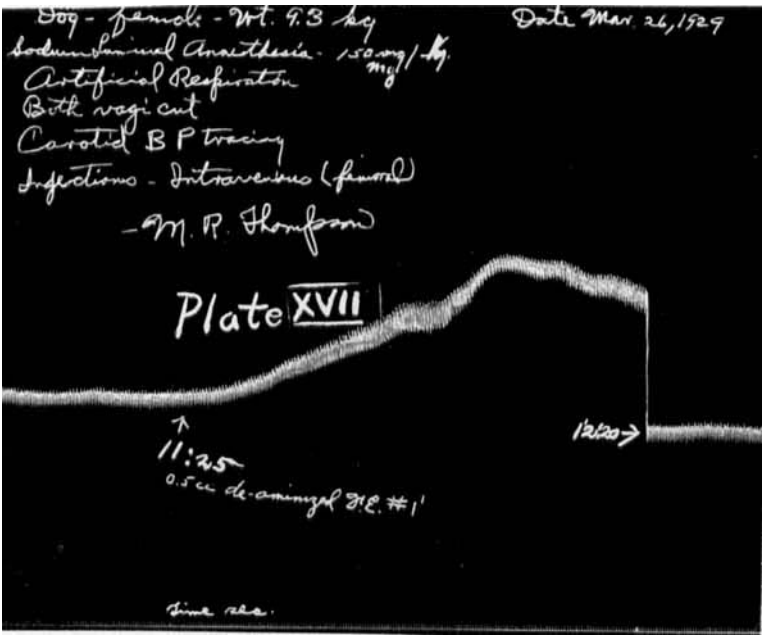

Plate XVII.—The pronounced and persistent rise in carotid blood pressure of a dog produced by the intravenous administration of 0.5 cc. of freshly prepared Purified Fluidextract of Ergot of Table VI. Note the absence of histamine effect. The response to a U. S. P. Fluidextract prepared from the same crude drug is shown in Plate XVI for comparison.

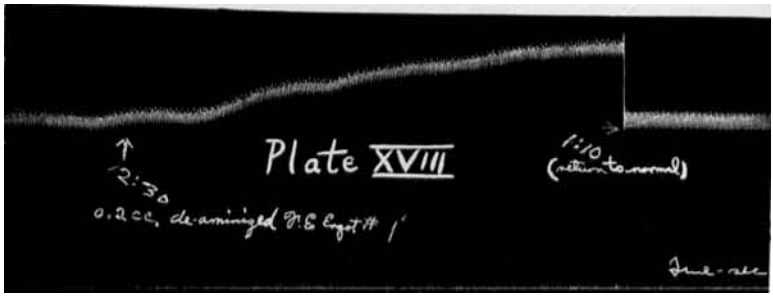

Plate XVIII.—The rise in carotid blood pressure produced by 0.2 cc. of Purified Fluidextract of Table VI. These Purified Fluidextracts produce an effect analogous to that of the pure alkaloid ergotamine exemplified in Plate VIII (41).

PHARMACO-DYNAMIC REACTIONS OF PURIFIED FLUIDEXTRACT OF ERGOT.

The pharmaco-dynamic effects produced by Purified Fluidextract of Ergot have been found to be entirely similar to those of pure solutions of either ergotamine or ergotoxine by the Cock's Comb, Isolated Guinea-Pig or Rabbit Uterus and Pressor Methods.

As set forth in preceding articles (41, 42), studies by the usual Pressor Methods to dogs or cats are especially significant in showing the qualitative nature of ergot preparations because of the marked differences in pressor effect produced by the most important of the non-specific amines, *i. e.*, histamine, and that produced by the specific ergot alkaloids. Therefore, experimental illustrations of pressor effects on anesthetized dogs only are included in this article to show the differences between U. S. P. Fluidextract of Ergot and "Purified or De-aminized Fluidextract of Ergot."

Plate XVI shows the effects produced upon the carotid blood pressure of an anesthetized dog by the intravenous administration of 0.5 cc. of the freshly prepared U. S. P. Fluidextract of Table VI. Note the similarity of this tracing and that of Plate XI of a preceding article (41) in which a mixture of the alkaloid ergotamine and histamine was given.

Plate XVII, in marked contrast to Plate XVI, shows the pressor effect produced in a similar experiment by 0.5 cc. of the "Purified Fluidextract" of Table VI. Note the similarity of this effect with that of pure ergotamine (Plate VIII, Part IV (41)), and the complete absence of histamine effect.

Plate XVIII shows the effect produced by the repetition of a smaller dose (0.2 cc.) of the "Purified Fluidextract" to the same dog as was used in Plate XVII.

Plate XIX illustrates the effect produced by the intravenous administration of the slightly alkaline watery extract from 0.5 gram of the drug entering into the preparation of the "Purified

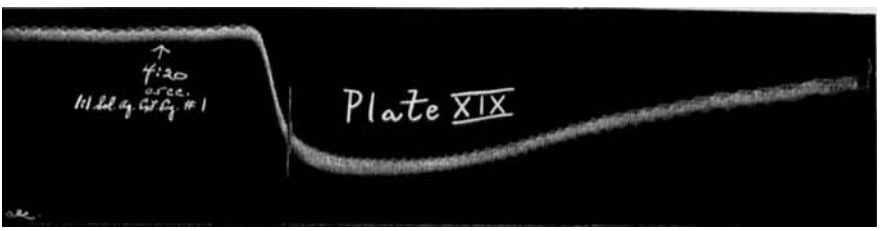

Plate XIX.—The effect produced upon the carotid blood pressure of a dog by the intravenous administration of an amount of the alkaline aqueous percolate representing 0.5 gram of the crude drug entering into the preparation of Purified Fluidextract of Ergot (Table VI). The effect is comparable in all respects to that produced by pure histamine (see Plate IX, Part IV (41)).

Fluidextract" of Table VI. The effect observed was entirely comparable to that produced by 0.5 mg. of pure histamine.

From these illustrations, it is seen that the U. S. P. Fluidextract owes its pressor activity to the non-specific amines *and* the alkaloids; that the "Purified Fluidextract" owes its activity to the alkaloids *only;* and that the preliminary alkaline aqueous percolation extracted essentially none of the active principles other than the undesirable non-specific amines, the most important of which is histamine.

THE BIOLOGICAL ASSAY AND STANDARDIZATION OF PURIFIED FLUIDEXTRACT OF ERGOT.

Preceding articles of this series (38, 39, 41, 42) bore experimental evidence showing that non-specific amines are frequently present in recently prepared Fluidextracts and Extracts of Ergot in sufficient concentrations to cause serious interference in the assay of these preparations for alkaloidal activity by the currently used bio-assay methods. Since the non-specific amines do not appear in the "Purified Fluidextract" herein described, this interference is not encountered. Therefore, several of the currently used bio-assay methods have been found satisfactory in estimating the alkaloidal activity of this preparation, as follows:

Method.	Accuracy. In terms of U. S. P. X potency.
Cock's Comb Method, U. S. P. X	±20%
Broom-Clark Isolated Rabbit Uterus Method (13)	±10%
Broom-Clark Isolated Rabbit Uterus Method, Modified (28)	±10%
Isolated Guinea-Pig Uterus Method (39)	±20%

THERAPEUTIC INDICATIONS AND USE.

The Purified Fluidextract of Ergot herein described contains approximately 45 to 50 per cent of alcohol in addition to approximately 0.5 to 0.6 per cent hydrochloric acid. It corresponds in physiological potency to Fluidextract of Ergot, U. S. P., and owes its entire activity to the specific alkaloids of ergot, being devoid of active non-specific amines such as histamine or tyramine. Therefore, this preparation is intended to be administered orally, in cases requiring ergot therapy, and in therapeutic dosage identical to that of Fluidextract of Ergot, U. S. P.

(*To be continued*)

TOOTH PASTES AND THEIR COMPOSITION.

Due to the active competition in the dentifrice industry, it "behooves the manufacturer to be sure of his ground and to pay strict attention to the quality of his product." A discussion is given of the substances in tooth paste, *e. g.*, abrasives, sweeteners and flavors, and liquid constituents, and their influence on the finished product. Soap is a valuable part of a good paste; it should be employed in finely powdered form, be made from high grade fats and oils, not contain more than 0.3% free alkali calculated as Na_2CO_3, and have a titer around 40–42° C., etc. The recently advanced claims as to the detoxification properties of sodium ricinoleate to replace germicides in the oral cavity have been shown by Leonard and others to require further verification. The importance of substantiating medicinal claims for tooth paste by actual experimental data is stressed. The U. S. Food, Drug and Insecticide Administration is devoting its attention to these medicinal claims. It has already ruled that no single medicine or combination of medicines known at this time can be regarded as effective as a preventive or cure for pyrorrhea (Riggs disease) or the common symptoms. Colloidal protection of tooth pastes is effected by the use of 0.5–3% gelatin, gums or chicle; 3% of free fatty acids; 5–10% of clays; or 50% of glycerite of starch.—J. P. in *Squibb Abstract Bulletin*, page A 1012.—E. G. Thomssen in *American Perfumer & Essen. Oil Rev.*, 24 (1929), 527.