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

PART IX. SUMMARY WITH CONCLUSIONS AND RECOMMENDATIONS.

BY MARVIN R. THOMPSON.*

The experimental results obtained in this investigation, together with a thorough review of the literature, have given rise to the following conclusions and recommendations regarding crude ergot, its constituents and its preparations:

CRUDE ERGOT.

I. VARIETIES AND COMMERCIAL SOURCE.

Practically all of the ergot of rye on the market is produced in Europe. Spain, Portugal, Russia and Poland furnish the greater part of the supply, although smaller amounts are obtained from more centrally located European countries. Ergot occurs in small quantities in rye fields of various localities of this country. It occurs casually in many sections of the cereal grain belt. The author is not aware of any attempts to utilize for medicinal purposes the amounts grown in the United States. Its growth has also been observed upon other members of the Graminiae, especially Quack or Couch Grass (*Agropyron repens*), and several varieties of wheat. The structure, size and appearance of ergot sclerotia obtained from grasses other than rye are characteristic for each variety, and differ markedly in size and shape from those of ergot of rye, although suitable extracts of wheat and couch grass ergot have been observed to contain significant potency when tested by the Cock's Comb Method.

Ergot of rye is the only variety used in medicine at the present time. In examining over two hundred samples it was observed that shipments obtained from Spain and Portugal usually contained a higher proportion of specific alkaloid than those obtained from Russia and Poland, although the majority of the samples of Russian and Polish ergot contained a significant amount of alkaloid, which equalled or exceeded the requirements of the U. S. P. No distinguishing pharmacological, toxicological or chemical characteristics other than differences in the average amounts of the specific alkaloid have been observed. The non-specific amines occur in variable quantities in the majority of lots of all varieties. Since the alkaloidal potency of Fluidextract of Ergot or other preparations must be standardized, Russian and Polish ergot of U. S. P. quality yield preparations which are equal in quality to those prepared from Spanish or Portuguese ergot. The Spanish and Portuguese varieties are to be preferred purely from the standpoint of alkaloidal content, in that a larger volume of U. S. P. Fluidextract is obtainable from a given weight of the ergot than when Russian or Polish ergot are used.

II. CONSTITUENTS.

The extractable constituents of ergot may be classified as follows:

(*1*) *Specific Alkaloids.*—Every sample of ergot so far examined has contained alkaloidal activity, thereby establishing the specificity of this activity for the drug. The value of ergot in medicine is due entirely to this specific alkaloidal activity. Depending largely upon the method employed, the following alkaloids can be isolated:

(*a*) Ergotoxine $C_{35}H_{41}O_6N_5$ (Barger and Carr (3)), identical to Amorphous Ergotinine (Tanret (2)), and Hydroergotinine (Kraft (4)) is an amorphous alkaloid and one of the most active of those yielded by ergot.

Crystalline salts can be prepared from this alkaloid.

(*b*) Ergotamine $C_{33}H_{35}O_5N_5$, a crystalline alkaloid first isolated by Stoll (60), equal in activity to ergotoxine. Ergotoxine and ergotamine are pharmaco-dy-

* Assistant Pharmacologist, Pharmacological Laboratory, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.

namically identical. Ergotamine is obtained in combination with solvent of crystallization and yields crystalline salts.

(*c*) Crystalline Ergotinine $C_{35}H_{39}O_5N_5$, first isolated by Tanret (2), is practically inactive.

(*d*) Ergotaminine, an isomer of ergotamine, also isolated by Stoll, has been shown to be practically inactive by Rothlin (61).

Ergotoxine, ergotamine and crystalline ergotinine have been isolated and studied during this investigation. It will be noted that the empirical formula of ergotamine differs from the equally active ergotoxine by C_7H_5OH, and that ergotoxine (active) differs from crystalline ergotinine (inactive) by one molecule of H_2O. Crystalline ergotinine is a dehydration product of ergotoxine, and the one can be readily converted to the other by chemical means. The crystalline alkaloids are invariably obtained in combination with solvents of crystallization.

Solutions of the active alkaloids of ergot are prone to rapid oxidation in the presence of air, which results in a loss of activity. Freshly prepared solutions are colorless. As the oxidation and loss in potency progresses, the solutions take on a yellowish tint which gradually changes to brown.

Ergotamine and ergotoxine are stable in the dry condition if protected from the air. Exposure to the air causes decomposition and loss in activity. If sealed in ampuls in a dry condition, their activity remains constant for many years. Both are entirely representative of the alkaloidal activity of ergot.

Although the solubilities of these alkaloids differ, both are soluble in many of the organic solvents such as ether, alcohol, acetone, etc., but are practically insoluble in water. They exhibit amphoteric reactions. Their solution in water may be affected by trituration with dilute solutions of organic acids (tartaric, citric, etc.) or by the addition of traces of mineral acids such as hydrochloric. A slight excess of hydrochloric acid causes precipitation, while a greater excess redissolves the precipitated alkaloid. Traces of some of the alkalies (hydroxides, carbonates, etc.) cause precipitation from aqueous solution, while an excess redissolves them. Many other electrolytes also cause precipitation.

The alkaloids mentioned above which exhibit any activity at all exert this activity synergistically. The alkaloidal activity of the numerous samples examined has been found to vary from 0.02 to 0.3 per cent (in terms of ergotamine or ergotoxine) as determined by biological methods. The majority of the samples examined contained between 0.05 and 0.150 per cent.

(*2*) *Non-specific Amines.*—Such proteinogenous amines as histamine, tyramine, acetylcholine, agmatine, guanido-butylamine, iso-amylamine, etc., have been identified with ergot by earlier investigators. No statement is available as to the proportions in which they exist in crude ergot. This investigation has furnished evidence showing that histamine is the only one of these substances which exists in ergot in sufficient amounts to cause significant pharmaco-dynamic activity. None of these amines contributes to the desirable clinical activity of ergot, and, therefore, they should be excluded from both oral and hypodermic ergot preparations, especially those intended for hypodermic use.

Histamine, or other amino bases, was not found to be present in all samples of ergot examined although the majority contained quantities varying from traces up to 0.15 per cent in terms of histamine (as determined by biological methods). More than 0.1 per cent was very unusual.

The non-specific amines are formed by enzyme action upon the otherwise inert proteinogenous constituents of the drug. It has been reported that such non-specific amines are not present in the freshly collected drug, thereby indicating that the amine formation takes place during storage rather than during the natural growth and development of the fungus upon the rye plant, although some old samples actually showing evidence of decomposition have proven to be devoid of active non-specific amines upon examination. That the non-specific amine activity of crude ergot may increase if stored in a damp condition, has been conclusively demonstrated in these studies. They are protein decomposition products and are not derived from the ergot alkaloids. There is no relationship between the amounts of non-specific amines and the alkaloids present in crude ergot. Such amine formation takes place in many plant and animal products under certain conditions due to the action of bacteria or proteolytic enzymes.

(*3*) *Pharmaco-dynamically Inert Extractives.*—These consist of one or more pigments or

coloring principles, 10 to 35 per cent of fixed oil, small amounts of inorganic salts, and appreciable quantities of complex proteinogenous substances. None of these substances lends to the desirable action of ergot preparations and should be excluded as much as possible.

III. THE CHEMICAL SEPARATION OF THE NON-SPECIFIC AMINE FRACTION FROM THE SPECIFIC ALKALOIDAL FRACTION OF CRUDE ERGOT AND FLUIDEXTRACT OF ERGOT.

Because of the complexities encountered in pharmacological studies of mixtures of the pharmaco-dynamically active constituents of ergot (as they occur in many of the popular types of ergot preparations), a chemical separation of the non-specific amine fraction from the specific alkaloidal fraction is necessary.

A method has been described for the preparation of a non-specific amine-free Fluidextract of Ergot which owes its activity exclusively to the specific alkaloids. The separation procedure can be successfully carried out as follows:

CRUDE ERGOT.

Method I.—Maceration and percolation of the powdered and de-fatted drug with an aqueous sodium bicarbonate menstruum and then percolation with water results in a percolate which contains the non-specific amine fraction of the drug. After the non-specific amines are removed (as indicated by testing upon the isolated guinea-pig uterus, or by pressor methods to cats or dogs), the specific alkaloidal fraction may be obtained by percolation of the amine-free drug with an acid-hydroalcoholic menstruum similar to that used for Fluidextract of Ergot, U. S. P.

Method 2.—Conversion of the drug to the U. S. P. X Fluidextract results in a preparation which, when freshly prepared, contains all of the non-specific amines *and* the specific alkaloids which were present in the parent drug. Separation of the two fractions of this preparation can be accomplished by the method described below for Fluidextract of Ergot.

FLUIDEXTRACT OF ERGOT, U. S. P. X.

This method of separation applies also to similar alcoholic orhydroalcoholic ergot preparations.

Method.—By completely de-alcoholizing a suitable portion of the preparation by concentration *in vacuo*, and bringing up to the original volume by the cautious addition of saturated sodium bicarbonate solution, the alkaloids are precipitated. After allowing the precipitate to settle, the clear liquid contains the non-specific amine fraction of the preparation. The amount of alkaloids which separates out serves as a rough indication of the alkaloidal activity of the preparation, although in old samples an appreciable amount of an inactive dark colored precipitate may result, thereby detracting from the significance of this test. After separating the precipitate by centrifugation, decantation or filtration, the non-specific amine activity (contained in the clear filtrate) may be studied. The specific alkaloidal precipitate may then be placed in solution for study by the cautious addition of 10 to 20 per cent of alcohol and a sufficient amount of hydrochloric acid to cause an acid reaction to the solution.

IV. THE PHARMACO-DYNAMIC BEHAVIOR OF THE ACTIVE CONSTITUENTS OF ERGOT UPON ORGANISMS AND TISSUES INVOLVED IN BIO-ASSAY METHODS.

According to pharmaco-dynamic activity and importance, the constituents of ergot may be conveniently divided into two groups; *1st*, the specific alkaloids; and *2nd*, the non-specific amines.

(*1*) *The Specific Alkaloids.*—Practically the entire alkaloidal activity of ergot is due to an alkaloid which, depending upon the method employed, can be isolated as either ergotoxine (amorphous ergotinine, hydroergotinine) or ergotamine. They are pharmacologically identical. The other alkaloids, if present at all, contribute little to the activity of ergot preparations.

(*2*) *The Non-specific Amines.*—As previously stated, the non-specific amine-activity of ergot, if present at all, is due almost entirely to histamine. The other amines, such as tyramine

and cholines, do not exist in sufficient amounts to exhibit significant activity in the doses and concentrations of ergot preparations usually employed in pharmaco-dynamic studies or in therapeutic practice.

Since many of the usual ergot preparations contain active amounts of histamine in admixture with the ergot alkaloids, the action of these active constituents in mixtures as well as individually will be summarized.

(A). COCKERELS: COCK'S COMB REACTION.

1. The Specific Ergot Alkaloids.—The alkaloidal activity of ergot is responsible for the well-known bluing or cyanotic effect of ergot preparations upon the comb of cockerels, the intensity of effect being proportional to dosage within maximum and minimum limits. The bio-assay method of the U. S. P. 10th Revision, depends upon this reaction, in which the ergot preparation is injected intramuscularly. Individual cockerels differ in their susceptibility to the ergot alkaloids, thereby making it absolutely essential that test cockerels be accurately standardized before attempts are made to utilize them in estimating the alkaloidal activity of ergot preparations. The susceptibility of cockerels to ergot alkaloids increases during continued usage. This, and natural changes, makes it necessary to re-standardize the test birds at least every three months. When properly applied, the Cock's Comb Method provides for the estimation of alkaloidal activity of ergot preparations within plus or minus 20% (in terms of U. S. P. X potency for fluidextract) unless interfering substances are present in the ergot preparation. Unless the "threshold reactions" of the test birds have been predetermined, this method is of qualitative value only. Minimum effective doses produce essentially no symptoms other than the bluing or blanching of the combs, wattels, tissue about the eyes, etc. Greater doses, in addition to the cyanotic effect, cause a drowsiness, slight salivation and an increase in the rate and depth of respiration. As the toxic dose is approached all of these symptoms are proportionately intensified. A marked general depression, salivation and comb cyanosis develops, and respiration becomes so rapid as to cause panting. Large doses (over 1.0 mg. of ergotamine, ergotoxine or 2.0 cc. of U. S. P. Fluidextract of Ergot) per Kg. may cause death by respiratory paralysis. The effect of the ergot alkaloids is very persistent. Depending upon the dose given and individual characteristics of the cocks, from one to ten days elapse before the combs return to normal. Although cockerels often return to apparent normality in several days, a shortening of the two weeks' rest period specified by the U. S. P. would not be advisable. Even when used every two weeks, morphological changes are produced. This is evidenced by loss in weight, abnormally rapid growth of the combs and an increase in susceptibility to the active constituents of ergot.

2. Histamine.—Aside from the specific alkaloids, histamine is the only constituent of ergot capable of producing a perceptible effect upon the cock's comb. Doses ranging from 0.2 to 0.6 mg. per Kg. produce a reddening of the combs, presumably due to capillary dilatation. No other symptoms become apparent and this effect passes off in less than two hours. Larger doses, ranging from 0.75 to 1.5 mg. per Kg., often produce a bluing or blanching of the combs similar to that caused by the ergot alkaloids, *especially in cockerels which have been subjected to ergot testing for an appreciable period.* The general symptoms induced by such doses of histamine differ greatly from those produced by the ergot alkaloids, the most important of which are marked depression, muscular weakness and tremors. Even in relatively large doses the effects are transitory, wearing off in several hours.

An actual manifestation of histamine symptoms is rarely encountered when testing Fluidextract of Ergot, but is frequently observed in testing aqueous pilular extracts which may contain high proportions of histamine but little or no alkaloid.

3. Mixtures of Ergot Alkaloids and Histamine.—Since the usual types of ergot preparations often contain the active principles in admixture, the effect produced by such mixtures is important.

Histamine frequently exists in crude ergot with the alkaloids in sufficient amounts to interfere with the comb reaction produced by the alkaloids when tested by the U. S. P. X method, which involves the application of the freshly prepared fluidextract. Although large doses of histamine alone often produce a bluing of the cock's comb, the amount of histamine present in the usual doses of any fluidextract is never sufficient to cause a perceptible bluing by itself. The amounts existing in a freshly prepared fluidextract are frequently sufficient in quantity, however,

to seriously interfere with the alkaloidal response of many cockerels. The interference is *destructive* rather than *constructive*, thereby causing such fluidextracts to exhibit an *apparent* alkaloidal potency which is lower than that actually present, unless an almost impossible number of birds are used for a single determination. Individual cockerels differ greatly with respect to this histamine interference. Using carefully standardized cockerels, this interference has been repeatedly demonstrated by the use of pure histamine in known mixture with ergotamine, and also by mixing alkaloid-free amine fractions of known histamine content with histamine-free fluidextracts of known alkaloid content.

It has been found to be a fact that any fluidextract prepared by the U. S. P. process contains as much as, or more than the quantity of ergot alkaloids indicated by the test, if, when assayed by the Cock's Comb Method, a satisfactory reaction is obtained from threshold doses of the preparation, regardless of the amine content. Any interference caused by the possible presence of interfering quantities of non-specific amines will result in a lower *apparent* alkaloidal potency than is actually present. This interference provides for a source of probable error in determining the alkaloidal potency of freshly prepared fluidextracts by this method, but such interference is rarely encountered in fluidextracts which are three or four months of age because of the rapid destruction of histamine in the menstruum of this preparation.

Aqueous Extracts of ergot, both liquid and pilular, may exhibit appreciable activity by the Cock's Comb Method even though practically no alkaloids are present. The observed effect is often due entirely to the histamine present. Histamine is more stable in such preparations than in the U. S. P. fluidextract.

(B). ISOLATED GUINEA-PIG UTERUS METHODS.

The effects of the various pharmaco-dynamically active constituents of ergot upon the isolated guinea-pig uterus depend to a great extent upon the condition of the tissue at the time the test is made. Immature virgin uteri are very sensitive and are stimulated to contraction by many substances, among which are the ergot alkaloids, histamine and tyramine. Repeated application of the ergot alkaloids results in diminishing response, while the response to the amines such as histamine and tyramine, is more constant. Histamine is by far the most powerful of the constituents of ergot in producing contraction. Therefore the effect observed from mixtures of these constituents (as found in many ergot preparations) is often due largely to the histamine present, but is also influenced by the alkaloids.

Pregnant uteri, or those taken while the animal is in œstrus are hyper-sensitive, and react in a manner similar to immature virgin strips. All such uteri are unsatisfactory for bio-assay purposes.

Mature guinea-pig uteri, preferably parous, have been found to be most suitable for studying the preparations and constituents of ergot. The action of the various constituents of ergot upon this type of isolated guinea-pig uterus is as follows:

1. *Histamine.*—Histamine is extremely active in producing prompt tonic contractions which are quite constant in repeated application. The effect is transitory, lasting only for several minutes. This substance is the most active of the non-specific amines of ergot, and is often responsible for the greater part of the contractions produced by the usual type of ergot preparations, even though significant amounts of alkaloids are also present.

2. *Tyramine.*—Tyramine is comparatively feeble in its effect. Therefore the small amounts occasionally occurring in ergot or its preparations are not sufficient to exhibit significant activity.

3. *Ergot Alkaloids.*—The active ergot alkaloids are practically ineffective in producing prompt tonic contraction in the usual concentrations. They exert a strong and persistent action, but the effect is one of inhibition or paralysis, rather than stimulation. The active alkaloids can be made to inhibit or totally abolish the contractions produced by histamine. The intensity of the effect of the alkaloids depends entirely upon the concentration and the time of contact. The effect is slow but very persistent. They increase the tonus and rhythmicity of spontaneous contractions.

4. *Mixtures of Histamine and Ergot Alkaloids.*—The application of mix-

tures of these substances, such as is present in many of the usual types of ergot preparations, cause prompt tonic contractions which are due to the histamine present. Repeated application of identical concentrations results in diminishing response due to the inhibiting or paralyzing effect of the alkaloids present in the mixture.

The action of histamine and of the ergot alkaloids, together with the principles involved, have been utilized in developing a method for the estimation of the non-specific amine activity and also a method for the estimation of the specific alkaloidal activity of ergot preparations using this type of isolated guinea-pig uterus. These methods have been described in Parts II and III of this series of articles.

(c). ISOLATED RABBIT UTERUS METHODS.

Strips of isolated rabbit uterus react to drugs in a manner decidedly different from guinea-pig uteri. The isolated rabbit uterus is involved in the well-known Broom-Clark Method for the estimation of the specific alkaloidal activity of ergot preparations. Most satisfactory uteri are obtained from mature rabbits, taken at a time when not in œstrus. Mature virgin or mono-parous uteri are usually satisfactory, but multiparae are best avoided.

1. *Epinephrine.*—Epinephrine, which produces relaxation of the isolated guinea-pig uterus, is extremely active in producing prompt tonic contractions of the isolated rabbit uterus by stimulation of the myoneural junctions of the sympathetic. Under proper conditions, these tonic contractions are constant in repeated application, and are similar in character to those produced by histamine on the guinea-pig uterus.

2. *Histamine.*—Histamine is comparatively feeble in its effect upon strips of rabbit uterus. Instead of causing prompt tonic contraction as it does upon the isolated guinea-pig uterus, the rabbit uterus is set into a series of rhythmic contractions of low magnitude, and this only from relatively high concentrations. Epinephrine is antagonistic to histamine and can be made to inhibit or completely abolish the response of the rabbit uterus to stimulant concentrations of histamine. At the same time, histamine in the presence of epinephrine, is capable of accentuating the epinephrine response, even if the histamine is of itself not present in sufficient concentration to stimulate response of the tissue, as first reported by Pattee and Nelson (28).

3. *Ergot Alkaloids.*—As in the case of the isolated guinea-pig uterus, the ergot alkaloids do not produce tonic contractions of the isolated rabbit uterus in the usual concentrations. They paralyze the sympathetic motor endings (myoneural junctions). Because of this effect, the ergot alkaloids are capable of inhibiting or completely abolishing the response of the tissue to epinephrine. This principle constitutes the basis for the Broom-Clark Epinephrine-Reversal Method for estimating the alkaloidal activity of ergot preparations. Histamine is capable of accentuating the epinephrine response. Therefore the presence of histamine in ergot preparations introduces a source of error. The effect of the ergot alkaloids is slow in developing, but is very persistent. The intensity of the reaction depends upon the concentration present and the time they are permitted to act. In carrying out the method, the time of action is limited to 10 minutes, even though the maximum intensity is not reached during this period. Pattee and Nelson (28) observed that any possible histamine interference could be obviated by replacing the drugged saline solution with fresh solution before observing the final epinephrine response. Such procedure removes any histamine without interfering with the alkaloidal effect. The Broom-Clark Method, as modified by Pattee and Nelson, has been found to be more accurate for estimating the alkaloidal activity of all types of ergot preparations than any other method proposed up to the present time.

(d). PRESSOR METHODS TO DOGS OR CATS.

Pressor methods to dogs or cats are invaluable in studying the activity of ergot in a qualitative way, but are not sufficiently accurate to be dependable for quantitative purposes. Of the

various constituents of ergot, only the active alkaloids and histamine have been found to be present in the general run of crude ergot samples in sufficient amounts to cause significant activity.

1. Histamine.—The blood pressure reaction of histamine is more powerful than that of any other constituent of ergot, although the effects produced by even relatively large doses are transitory. Injected intravenously, histamine produces a sharp fall in blood pressure due to vaso-dilatation, which is followed by a distinct manifestation of cardiac inhibition as evidenced by a decrease in amplitude of pulsations. The blood pressure and heart action return to apparent normality in approximately five minutes, unless the dose is unnecessarily large. Oral administration of histamine or the non-specific amine fraction of ergot produces no pressor or depressor effects.

2. Tyramine.—Tyramine produces a rise in blood pressure but has not been observed to occur in ergot or ergot preparations in sufficient amounts to produce this effect from reasonable doses given hypodermically. The predominance of histamine effect invariably masks that which might be caused by the presence of traces of tyramine.

3. Ergot Alkaloids.—Small doses of the ergot alkaloids produce a slow, but very persistent rise in blood pressure due to vaso-constriction. The induced rises in blood pressure diminish with repeated dosage, due to vaso-motor paralysis, until a condition is reached such that either no effect is produced upon the blood pressure at all, or a fall in pressure results which cannot be raised by epinephrine. A single large dose of the ergot alkaloids can also be made to abolish or reverse the usual effect of epinephrine upon cats but is very difficult to produce in dogs. This phenomenon constitutes the well-known "Vasomotor-reversal" and has been suggested as a method for determining the alkaloidal activity of ergot preparations. The amount of ergot alkaloids necessary to produce this reversal varies greatly with individual dogs or cats, thereby preventing even reasonable accuracy as a quantitative method. Only one trial is possible upon a single animal because of the great persistence of alkaloid effect, thereby preventing a comparison of an unknown preparation with a suitable standard.

4. Mixtures of Histamine and Ergot Alkaloids.—Histamine produces an effect which is antagonistic to that produced by the ergot alkaloids. The injection of these substances in mixture (such as is present in some of the usual ergot preparations) results in a prompt fall in blood pressure due to the histamine present, which is followed by a rise in pressure due to the alkaloid. The presence of histamine in the mixture prevents a full manifestation of alkaloidal effect because of the power of histamine in producing vaso-dilatation, cardiac inhibition, and perhaps increased permeability of capillaries. Histamine has been found to exist in ergot in sufficient amounts to be capable of actually reversing the usual effect of the ergot alkaloids, *i. e.*, an effective dose of ergot alkaloids, if preceded by an effective dose of histamine, produces a fall in pressure instead of the usual rise, even though the dose of the alkaloid is not given until the fall in pressure which follows the administration of the histamine has returned to normal. Because of the persistent paralyzing effect of the ergot alkaloids, the marked interference caused by histamine, and the variation in susceptibility exhibited by individual test animals, pressor methods are adaptable in determining the activity of ergot preparations in only a qualitative way.

V. STABILITY AND PRESERVATION OF CRUDE ERGOT.

Whole or crude ergot is remarkably stable if it is stored in a thoroughly dry condition. In such condition, the temperature does not play an important part. Insect infestation can be avoided by keeping in a dry condition, preferably in a cold place, and by the proper use of naphthalene, carbon tetrachloride, carbon disulphide or chloroform.

When stored in a damp condition, very objectionable changes rapidly take place, the rapidity depending upon the amount of moisture present and the tempera-

ture. The "Acid Number" of the fixed oil increases due to the development of rancidity, the alkaloidal activity decreases, and the non-specific amine activity often increases. As already stated, no relationship exists between the decrease in alkaloidal activity and the increase in non-specific amine content. An appreciably damp condition also increases the liability of insect infestation and causes a rapid growth of mold.

Powdered ergot is less stable than whole ergot unless the fixed oil is extracted before storage. Under proper storage conditions, crude ergot or powdered and defatted ergot can be kept for many years without undergoing serious loss in activity or other objectionable changes.

The samples of better quality (those exhibiting the greatest amount of alkaloidal activity and best condition) exhibit a short, corky fracture and evolve a pleasant aromatic odor. The fractured surfaces of the sclerotia are white or yellowish white and are not glassy-smooth. They are reduced to a brown or grayish brown powder of a rather soft, mealy texture with comparative ease.

Poorer quality samples exhibit a tough, horny fracture and are either practically devoid of odor or evolve a soapy, unpleasant odor. The fractured surfaces of the sclerotia are usually tinted with a brown, blue or purple color and are usually very smooth. They are reduced to a purplish gray powder with greater difficulty than samples of good quality. The resulting powder is composed of more or less sharp, angular fragments and is of a rough texture. The size and general shape do not constitute significant criteria as to the quality, although large grains are usually preferable.

ERGOT PREPARATIONS.

A very large number of different varieties of commercial ergot preparations are available upon the drug market at the present time. Practically all of these different preparations fall into one of three general classes, according to the method of extraction involved, as follows:

1. *Liquid Products* prepared by extraction of the comminuted drug with an alcoholic, hydro-alcoholic or acid-hydro-alcoholic menstruum, such as Fluidextract of Ergot, U. S. P., 10th Revision.

2. *Aqueous Extracts.*

(*a*) Liquid products prepared by extraction of the comminuted drug with an aqueous menstruum, such as Liquid Extract of Ergot, B. P. Many of the preparations of this type are purified by concentrating and precipitating some of the extractives by the addition of alcohol.

(*b*) Solid, semi-solid or pilular extracts, prepared by extraction of the comminuted drug with an essentially aqueous menstruum and concentrating to a pilular consistence, such as Aqueous Extract of Ergot, N. F., 5th Edition, and Extract of Ergot, B. P.

3. *Preparations Composed of or Containing the Pure Isolated Constituents of Ergot.*

The pharmaco-dynamic activity exhibited by these different classes of ergot preparations differs so greatly that one would hardly believe that the same drug had been involved in their manufacture. The nature and amount of activity of freshly prepared ergot preparations depend upon the condition of the crude drug, and the menstruum and technique used in the extraction process, many being practically inactive even when freshly prepared. None of these liquid or semi-solid products is absolutely stable, although some are more so than others. The rate of deterioration in any case depends upon the composition of the menstruum or diluent, exposure to air and light, and the storage temperature.

The medicinal value of any ergot preparation is wholly dependent upon its specific alkaloidal activity. The non-specific amines have no medicinal importance and are of significance only because of the effects produced in experimentation involving the usual pharmaco-dynamic or bio-assay methods.

CLASS (1). PREPARATIONS INVOLVING THE EXTRACTION OF THE DRUG WITH AN ALCOHOLIC, HYDRO-ALCOHOLIC OR ACID HYDRO-ALCOHOLIC MENSTRUUM.

The Fluidextract of Ergot of the U. S. P., 10th Revision, has been found to be the most satisfactory of available ergot galenicals. The prescribed process of manufacture results in a product which, when freshly and carefully prepared, contains practically all of the specific alkaloids and the non-specific amines which were present in the parent drug. Any non-specific amine activity is usually lost within a period of three or four months from the time of manufacture. The hydrochloric acid of the prescribed menstruum plays a double rôle. *First*, it increases the efficiency of the menstruum in extracting the specific alkaloids, and *second*, it increases the stability of the product. Proper control of this acidity is therefore of vital importance. The use of organic acids, such as citric or tartaric, in place of hydrochloric serves no good purpose, in that such departure from the U. S. P. method decreases the efficiency of the menstruum in extracting the alkaloids and also detracts from the stability of the finished product. A menstruum consisting of alcohol or diluted alcohol without acid is less efficient for extracting the ergot alkaloids than one which contains acid and in addition seriously detracts from the stability of the product. Since the ergot alkaloids are unstable in the presence of air and warm temperatures, it has been found distinctly advantageous to concentrate the exhaust percolate (obtained after the reserve portion) at a low temperature under reduced pressure.

(*a*) *Stability of Fluidextract of Ergot, U. S. P.*—This preparation appears to be the most satisfactory of available ergot galenicals from the standpoint of stability, although it is now definitely known that even this preparation is prone to deterioration, regardless of the precautions ordinarily taken in attempts at stabilization.

The non-specific amine activity which may or may not be present, is due almost entirely to histamine. It rarely occurs in commercial fluidextracts or other fluidextracts which have attained an age of more than six months, because of its lability in the medium of this preparation. The deterioration of this amine activity begins immediately at the time of manufacture, and progresses so rapidly that practically none remains after three or four months, regardless of the storage conditions under which the fluidextract is kept.

The specific alkaloidal activity of Fluidextract of Ergot to which the medicinal value of the preparation is due, fortunately is more stable than the non-specific amine activity, but nevertheless, is prone to deterioration. Exposure to air and warm temperatures hasten this loss of activity. Unnecessary loss in activity may be avoided by keeping in well-filled, tightly stoppered containers in a refrigerator or cold-room, or by replaing the air above the liquid in the container with an inert gas such as carbon dioxide or nitrogen. Under such conditions the greater part (approximately two-thirds) of the alkaloidal activity is retained for a period of one year. Frequent opening of the containers, as in dispensing, causes a marked increase in the rate of deterioration. For this reason the capacity of containers used in the distribution of this product to hospitals and dispensing pharmacists should in no case exceed four ounces, while one ounce containers would be even more satisfactory for placing a dependable fluidextract at the disposal of the practicing physician.

Sealing Fluidextract of Ergot in ampuls *in vacuo* provides for a greater degree of stability than other methods of keeping. By such procedure, and storing the sealed ampuls in a cold place, the greater part of the activity is retained for two years.

(*b*) *Bio-Assay Standards for Fluidextract of Ergot.*—An extensive study of the present U. S. P. X Standard Fluidextract of Ergot has revealed that its potency is not constant. Aging this Standard for six months prior to sealing in ampuls, as specified in the U. S. P. is distinctly advantageous. It causes a complete disappearance of non-specific amine activity and provides for a greater degree of stability of the all-important alkaloidal activity since the most rapid de-

terioration takes place during a two- to three-month period immediately following manufacture. Unfortunately, even if careful attention is given to the details outlined in the U. S. P. for the preparation of this Standard, *i. e.*, aging, sealing in hard-glass ampuls *in vacuo*, etc., the resulting product is not absolutely stable. This Fluidextract, therefore, does not constitute an ideal bio-assay Standard for Ergot or Fluidextract of Ergot unless the alkaloidal potency is determined at least every three months by accurate comparison with a standard of constant potency such as ergotamine tartrate, and replacing lots showing deterioration with a new lot of Standard Fluidextract as soon as loss in activity is evident. Only under such conditions would constant potency of the U. S. P. X Standard Fluidextract be insured.

Ergotamine Tartrate $(C_{33}H_{35}O_5N_5)_2.(H_2C_4H_4O_6)$, a crystalline salt of one of the most active of the alkaloids of ergot, has been shown to be entirely representative of the desirable activity of ergot and stable under conditions easily provided. It has been successfully used as a bio-assay standard during this entire investigation. When used in this capacity, the solution must be freshly prepared as required. Ergotamine tartrate is only sparingly soluble in water. The addition of a few drops of dilute hydrochloric acid to the solvent causes imemdiate and complete solution. It has been the practice of the author to prepare a 1 : 1000 solution, then using portions of this to prepare suitable dilutions for experimental application.

CLASS (2). AQUEOUS EXTRACTS OF ERGOT.

(*a*) *Liquid Aqueous Extracts.*—This class of ergot preparations includes those which involve extraction of the drug with essentially an aqueous menstruum and which are marketed in a liquid form. Liquid Extract of Ergot, B. P., as well as a great number of proprietary specialties fall into this class. Many of these aqueous preparations are subjected to processes of purification for the purpose of rendering them suitable for either hypodermic or oral administration.

This investigation has revealed that extraction of the drug with water removes the non-specific amines and appreciable amounts of inert material, but that no significant amounts of the all-important ergot alkaloids appear in the finished product. Any activity exhibited by such preparations is usually due principally to histamine.

The addition of alcohol to the extraction menstruum in amounts ranging from 10 to 50 per cent causes the removal of somewhat greater proportions of the alkaloids, although such menstrua are far less efficient than that of the U. S. P. which contains 2 per cent of hydrochloric acid, U. S. P., in diluted alcohol. Aqueous or hydro-alcoholic menstrua without acid are not only inefficient in extracting the specific alkaloids, but the products obtained by extraction with such solvents are so unstable as to be of no value.

The pharmaco-dynamic activity of such preparations is due almost entirely to exceedingly variable quantities of histamine, which substance is of no value in ergot preparations since it is not responsible for any of the therapeutic virtues of ergot.

(*b*) *Solid, Semi-Solid or Pilular Aqueous Extracts.*—This class includes preparations such as Extract of Ergot, B. P., Aqueous Extract of Ergot, N. F., 5th Edition, the "Ergotins," "Ergotines," etc., which occur on the market as such or in combination with other ingredients in pills or tablets.

These solid or pilular extracts are usually prepared essentially according to the method described in the B. P. for Extract of Ergot or in the N. F. for Aqueous Extract of Ergot. These methods involve extraction of the drug with an aqueous menstruum, and, therefore, result in preparations which are practically devoid of ergot alkaloids, and are therefore worthless. Varying amounts of histamine usually account for any pharmaco-dynamic activity observed. This substance is more permanent in liquid or semi-solid extracts than in the U. S. P. X Fluidextract.

CLASS (3). PREPARATIONS COMPOSED OF, OR CONTAINING THE PURE ISOLATED SPECIFIC ALKALOIDS OF ERGOT.

The important specific alkaloids ergotamine and ergotoxine (otherwise known

as hydroergotinine or amorphous ergotinine), to which the therapeutic value of ergot is due, are now available upon the drug market in the form of their dry salts or in solution. The solutions are subject to deterioration when exposed to air and warm temperatures. The conditions and factors which influence the rate of deterioration of these solutions are, in general, the same as those described under Fluidextract of Ergot. Under proper conditions they are reasonably stable. The dry alkaloids or their salts are stable over a period of many years if they are sealed in ampuls or are otherwise fully protected from air and moisture. In the presence of air and moisture, the dry substances assume brownish tints which are indicative of a loss in potency.

In the first of this series of articles, it was pointed out that ergot preparations available to physicians were highly unsatisfactory, and five possible reasons for this condition were enumerated. In the light of the information yielded by this investigation, it is believed that faulty methods and technique of manufacture, together with exposure of these preparations to improper storage conditions for almost unlimited periods of time before use, are responsible for the unreliability of ergot preparations more than any other factors.

THE CHOICE OF METHODS FOR THE BIOLOGICAL ASSAY OF CRUDE ERGOT AND ITS PREPARATIONS.

Because of the variability in activity exhibited by individual lots of crude ergot, uniformity in resulting preparations can be obtained only by standardization of the finished products. Standardization is possible only by the application of an assay method which measures the therapeutically active constituents. Since the specific alkaloidal activity is wholly responsible for the medicinal value of any ergot preparation, the method of assay must measure only the specific alkaloidal activity, irrespective of any other inert or pharmaco-dynamically active principles which may be present.

A number of chemical and biological assay methods for estimating the alkaloidal content or activity of ergot preparations have been reported during recent years, and shown to yield dependable results, at least in the hands of their originators.

Since a number of these methods are now available, the choice of a method for general use in assaying and standardizing the alkaloidal activity or content of ergot and its preparations resolves itself into a consideration of accuracy or dependability as evidenced by wide usage in the hands of the greatest possible number of investigators.

Up to the present time, no chemical methods have shown sufficient accuracy or dependability in extensive application to receive wide-spread recognition. Of the numerous biological methods now available, only two have received sufficient recognition in extensive application to warrant consideration. These are the well-known Cock's Comb Method, now official in the U. S. P., and the Epinephrine-Reversal Method involving the isolated rabbit uterus. The latter method was originated by Broom and Clark in 1922, and has since been studied and applied successfully by a host of investigators in this country and abroad. These workers have invariably found that the two methods yield similar results but that the Rabbit Uterus Method provides for somewhat greater accuracy than the U. S. P. Method. The evidence obtained in the author's studies have confirmed this conclusion. The chief objection to the Rabbit Uterus Method, as expressed by the various workers, is that it is more difficult of technique and more time-consuming.

The results of this investigation have shown that assays of various types of ergot preparations by the Cock's Comb Method are not always dependable, due to the occasional presence of interfering quantities of non-specific amines and to changes in the susceptibility and response of

individual test cockerels. Even in the complete absence of interfering pharmaco-dynamically active substances, as in the case of fluidextracts which have been aged several months, causing a disappearance of non-specific amine (histamine) activity, the Rabbit Uterus Method has been found to be more accurate and more dependable than the Cock's Comb Method in estimating the alkaloidal activity of all types of ergot preparations, regardless of how carefully every detail of technique is observed in the application of the Cock's Comb Method. In assaying crude ergot by the Cock's Comb Method, which necessitates the application of a freshly prepared fluidextract, the occasional presence of high proportions of histamine cause such marked interference that an almost impossible number of birds must be used on a single sample in order to attain even a reasonable degree of accuracy.

THE POTENCY OF FLUIDEXTRACT OF ERGOT.

Since ergotamine and ergotoxine have become available, an appreciable amount of published and unpublished information upon the clinical or therapeutic application has established that an average oral dose of either of these alkaloids, or its equivalent in an ergot preparation must approximate 1.0 mg. to be effective.

This investigation has shown that it is possible to prepare Fluidextract of Ergot containing 0.05 per cent of ergot alkaloids (in terms of either ergotamine or ergotoxine as determined by biological methods) from the average crude ergot now available. A fluidextract of this potency contains the equivalent of 1.0 mg. of ergotamine or ergotoxine in the present U. S. P. dose of 2.0 cc.

The author concludes, therefore, that this constitutes a suitable potency for Fluidextract of Ergot.

TOXICOLOGICAL ASPECTS OF ERGOT AND ALKALOIDAL PREPARATIONS OF ERGOT.

The only constituents of ergot, regardless of the natural condition of the drug, which occur in sufficient amounts to be capable of producing either acute or chronic toxicological symptoms by oral administration are the active specific alkaloids, upon whose presence the therapeutic value of ergot depends. The ergot alkaloids are active by either oral or hypodermic administration.

The effects of an adequate dose of the active ergot alkaloids persists for a period varying from one hour to one day or more, depending upon the dose and the individual susceptibility or condition of the patient. This must be taken into consideration to avoid the possibility of producing toxic symptoms due to a cumulative action, when such preparations are applied therapeutically in repeated dosage at short intervals. They are responsible for the gangrenous symptoms frequently observed in experimental animals. These symptoms can be produced only by the administration of repeated doses at short intervals over a considerable period. If the prescribed two weeks' rest period is observed on cockerels in applying the U. S. P. method of assay, gangrene rarely occurs. This condition frequently develops, however, if the period between doses is appreciably shortened. The same applies to other subjects as well as cockerels.

Although a number of other non-specific amines may occur casually in insignificant amounts, histamine quite often exists in crude ergot and some of its preparations in sufficient amounts to exhibit appreciable pharmaco-dynamic activity. None of these amines or amino-bases play a part in the therapeutic action or toxicology of crude ergot or oral ergot preparations, because of the fact that they are totally inactive by oral administration. Even by the hypodermic administration of amounts of histamine comparable to those occurring in the usual doses of ergot preparations, the effects produced, although prompt, are exceedingly transitory. These non-specific amines serve no good purpose in either oral or hypodermic preparations, however, and should be excluded from all preparations as much as possible, but more especially from those intended for hypodermic use.

RECOMMENDATIONS.

1. *Storage of Ergot.*—Crude ergot should be stored in a thoroughly dry condition and in an absolutely dry place at all times.

Powdered ergot should be de-fatted before storing for any appreciable period, to avoid the development of rancidity and unnecessary loss in potency.

Ergot is subject to insect infestation under any conditions of storage but is most likely to occur if a damp condition prevails. The judicious use of naphthalene, carbon tetrachloride, chloroform, etc., and storing in a dry, cold place can be made to adequately control this condition.

2. *Method of Assay of Crude Ergot and Fluidextract of Ergot U. S. P.*—Because of its superiority over the present official method of assay with respect to accuracy and dependability in estimating the specific alkaloidal activity, it is recommended that the "Epinephrine-Reversal," otherwise known as the "Isolated Rabbit Uterus Method" be adopted in the next Pharmacopœia in place of the now official Cock's Comb Method, for the biological assay of Ergot and Fluidextract of Ergot.

3. *Biological Assay Standard.*—Because of variability in susceptibility of individual test objects the application of any biological assay method for Ergot or its preparations necessitates the use of a standard preparation for comparison. This standard preparation must be constant in potency. In the light of results obtained in this investigation only two possibilities remain, either of which would prove a satisfactory standard, as follows:

(*a*) The use of Crystalline Ergotamine Tartrate, containing 84.5 per cent of ergotamine base, to be distributed to manufacturers as such, or in the form of the freshly prepared solution, sealed in ampuls in vacuum and bearing an expiration date, or

(*b*) The use of the Standard Fluidextract of U. S. P. X but which is accurately adjusted to a definite potency by comparison with ergotamine tartrate and then sealed in ampuls in vacuum. As soon as a given lot shows deterioration, to be determined by frequent check assays, a new, accurately standardized lot would be pressed into use. As in (*a*), an expiration date must appear on the label.

It is recommended that one of these two alternatives be adopted in the next Pharmacopœia.

4. *The Potency of Ergot and Fluidextract of Ergot.*—Because of the now known posology of ergot alkaloids, and the alkaloidal potency shown by crude ergot now available, it is recommended that a potency equivalent to not less than 0.05 per cent of ergot alkaloids in terms of ergotamine base be adopted as the minimum for crude ergot.

It is recommended that a potency equivalent to not less than 0.04 per cent and not more than 0.06 per cent of ergot alkaloids in terms of ergotamine base be adopted for Fluidextract of Ergot.

5. *Expiration Date for Fluidextract of Ergot.*—Because of the fact that Fluidextract of Ergot is prone to deterioration, and to prevent inactive fluidextracts from falling into the hands of physicians, it is recommended that an expiration date be required to appear upon the label, and that this expiration date be such that the fluidextract will not be used for medicinal purposes for more than one year from the date of final assay and standardization, or, that the standardization of potency shall be such that the alkaloidal activity will not be less than 0.04 per cent of ergot alkaloids in terms of ergotamine base, upon the date of expiration.

6. The Containers for Distributing Fluidextract of Ergot.—Because exposure to air is the most important factor in causing deterioration of Fluidextract of Ergot, and because warm temperature and light hasten the destruction of the ergot alkaloids, it is recommended that the next U. S. P. require that this preparation be kept in a cold place in tightly stoppered, non-metallic containers, filled as nearly completely, as practicable, carefully protected from light, and distributed to retail pharmacists in original amber-colored glass containers having a capacity of not more than four fluidounces.

7. Improving the Quality and Stability of Fluidextract of Ergot by Aging.—Since the most rapid deterioration of the alkaloidal activity of Fluidextract of Ergot occurs during a period of one to three months immediately following manufacture and since aging in a cold room further provides for the elimination of undesirable non-specific amine activity and the precipitation of variable amounts of an inactive sludge which should be discarded, it is recommended that the next revision of the U. S. P. require that Fluidextract of Ergot be aged for three months in completely filled tightly-stoppered, non-metallic containers in a cold place protected from light before standardizing and packaging in small containers for distribution.

8. The HCl Content of Fluidextract of Ergot.—Because of the great influence exerted by the presence of hydrochloric acid in increasing the efficiency of the extraction menstruum in extracting the specific alkaloids from the powdered drug, and in stabilizing the alkaloidal activity in Fluidextract of Ergot, the amount of hydrochloric acid used should be very carefully controlled, both during the process of extraction and in the finished product.

9. Rancidity of Crude Ergot.—The "Acid Number" of the fixed oil of ergot is an index of rancidity, and can be determined by a method described in Part VII of this series of articles. An "Acid Number," as defined in this article, of more than 15 shows an objectionable degree of rancidity, and indicates that the drug had attained great age, or that it had seriously suffered from improper storage conditions.

It is recommended that this test for rancidity be adopted in U. S. P. XI and that a maximum "Acid Number" of 15 be permissible for crude or powdered ergot.

10. Aqueous Extract of Ergot, N. F., 5th Edition.—Because the method of preparation specified by the N. F., 5th Edition, for Aqueous Extract of Ergot, has been found to result in a product which is practically devoid of specific ergot alkaloids, regardless of the alkaloid content of the parent drug, it is recommended that this or any other preparation not containing significant and defined amounts of specific alkaloidal activity be omitted from succeeding editions.

The author acknowledged indebtedness to Messrs. W. T. McClosky and Nicholas G. Barbella of this laboratory for valuable assistance rendered during these studies; to Drs. P. B. Dunbar, J. J. Durrett and Mr. A. G. Murray of the Food Drug and Insecticide Administration for the reading and criticism of manuscripts; and to the Sandoz Chemical Co. and Burroughs, Wellcome & Co. for a generous supply of specific ergot alkaloidal salts.

REFERENCES.

(1) Nelson and Pattee, *Am. Jour. Obst.*, 16 (1928), 73.
(2) Tanret, *Compt. rend. acad. sci.*, 81 (1875), 896.
(3) Barger and Carr, *Chem. News*, 44 (1906), 89.

(4) Kraft, *Arch. Pharm.*, 244 (1896), 336.
(5) Spiro and Stoll, *Schweiz. Med. Wochschr.*, 51 (1921), 525.
(6) Barger and Dale, *Biochem. J.*, 2 (1907), 240.
(7) Kobert, *Arch. exptl. path. Pharmakol.*, 18 (1884), 316.
(8) Simonnet and Tanret, *Compt. rend. acad. sci.*, 182 (1926), 493.
(9) Stoll and Rothlin, *Schweiz. Med. Wochschr.*, 57 (1927), 106.
(10) Dale and Spiro, *Arch. exptl. Path. Pharmakol.*, 95 (1922), 337.
(11) Kutscher, *Centr. Physiol. Chem.*, 24 (1910), 163.
(12) Rothlin and Schlegg, *Wien. med. Wochschr.*, 75 (1925), 2018–2109.
(13) Broom and Clark, *J. Pharmacol. and Exp. Ther.*, 22 (1923–1924), 59.
(14) Sollmann, "Man. Pharmacol.," Edit. III, 457.
(15) Gittinger and Munch, JOUR. A. PH. A., 16 (1927), 505.
(16) Forst and Weese, *Arch. exptl. Path. Pharmakol.*, 117 (1926), 232.
(17) Carr and Dale, "Year Book of Pharmacy" (1903), 505.
(18) Nelson, *J. Lab. Clin. Med.*, 8 (1923), 318.
(19) Clark and Broom, *Pharm. J. & Pharmacist*, July 28, 1923.
(20) Dale and Laidlaw, *J. Pharmacol. and Exp. Ther.*, 4 (1912–1913), 75.
(21) Smith and McClosky, "Public Health Reports," *Reprint 822* (Mar. 16, 1923).
(22) Wood and Hofer, *Arch. Int. Med.*, 6 (1910), 388.
(23) The National Formulary, 5th Edition (1926).
(24) The U. S. Pharmacopœia, 10th Revision.
(25) "Report of Council of Pharmacy and Chem." of Am. Med. Ass'n. (May 4, 1929).
(26) Dixon, "Man. Pharmacol.," 5th Edition, 211.
(27) A. Harmsma, *Pharm. Weekbl.*, 65 (1928), 1114.
(28) Pattee and Nelson, *J. Pharmacol. & Exp. Ther.*, 36 (1929), 85.
(29) Crawford & Crawford, *J. A. M. A.*, 61 (1913), 19.
(30) Edmunds & Hale, *Hygienic Lab. Bulletin*, 76 (July 1911).
(31) Rothlin, *J. Pharmacol. & Exp. Ther.*, 36 (Aug. 1929), 657, No. 4.
(32) Planelles, *Arch. exptl. Path. Pharmakol.*, 105 (1925), 38.
(33) Mendez, *J. Pharmacol. & Exp. Ther.*, 32 (1927–28), 451.
(34) Swanson, JOUR. A. PH. A., 18 (1929), 1127.
(35) Barger, "Zur Geschichte des Mutterkorns;" *Naunyn-Schmiedebergs Arch.*, 138 (1928), 105–119.
(36) Van Urk, *Pharm. Weekbl.*, 66 (1929), 473–481.
(37) Thompson, Part I, JOUR. A. PH. A., 18 (1929), 1106–1111.
(38) Thompson, Part II, *Ibid.*, 18 (1929), 1111–1118.
(39) Thompson, Part III, *Ibid.*, 18 (1929), 1118–1124.
(40) British Pharmacopœia (1914).
(41) Thompson, Part IV, JOUR. A. PH. A., 19 (1930), 11–23.
(42) Thompson, Part V, *Ibid.*, 19 (1930), 104–117.
(43) Perla and Marmorston-Gottesman, *J. Physiol.*, 89 (1929), 152, No. 1.
(44) E. Geiger, *Arch. exptl. Path. Pharmakol.*, 146 (1929), 109.
(45) Weiss, Ellis and Robb, Reported at XIIIth International Phys. Congress at Boston, Aug. 19, 1929.
(46) Mammoser, Albi and Boyd, Reported at XIIIth International Phys. Congress at Boston, August 19, 1929.
(47) "Woodman's Food Analysis," 2nd Edit., page 416.
(48) Thompson, Part VI, JOUR. A. PH. A., 19 (1930), 221–228.
(49) Thompson, Part VII, *Ibid.*, 19 (1930), 436.
(50) Barger and Carr, *J. Chem. Soc.*, 91 (1907), 337.
(51) Tanret, *J. pharm. chim.*, 24 (1906), 397.
(52) Stoll, *Arch. exptl. Path. Pharmakol.*, 138 (1928), 111.
(53) Forst, *Ibid.*, 114 (1926), 125.
(54) Burn and Ellis, *Pharm. J.*, 118 (1927), 384.
(55) Liptak, "Ber. d. ungar. Pharm. Ges." (1926), S. 286–287. Abstract: *Heil- Gewürz-Pflanz.*, 10 (1927), 109.

(56) Prybill and Maurer, *Arch. Pharm.*, 7 (1928), 464.
(57) Wokes, *J. Pharm. and Pharmacol.*, II (1929), No. 3, 384.
(58) *British Medical J.* (Nov. 23, 1929), 955.
(59) Bourne and Burn, *J. Obst. and Gyn. of British Empire* (1927), Vol. 34; No. 2.
(60) Stoll, "Verh. d. Schweiz. Nat. Ges." (1920), *Schweiz. Apoth. Ztg.*, 60 (1922), No. 26.
(61) Rothlin, *Arch. expil. Path. Pharmakol.*, 138 (1928), 115.

STANDARDIZATION AND DETERIORATION OF RENNIN.*

BY L. D. HAVENHILL.

Early this spring while removing some drugs from a storage cabinet, I uncovered some samples of rennin on which considerable work was done in 1912. Dr. Klein in his report on Digestive Ferments and Glandular Products (4) finds that the "N. F. method as an absolute means of estimating rennin activity is unreliable" and suggests the possibility of adopting a standard rennin for control purposes. It therefore occurred to me that a reëxamination of these would furnish useful information concerning their deterioration, and at the same time afford an opportunity to check upon the N. F. V method of assay.

When these samples were originally assayed, fresh, whole milk from a mixed herd and averaging 3.5% butter-fat by Babcock test was used. No serious discrepancies in the time of coagulation for the daily samples of milk were observed.

STANDARDIZATION OF MILK.

Before reassaying these samples, a series of daily assays on milk was made using a new sample of powdered rennin—Rennin No. 12, of Table III. The results of these assays are given in Table I.

TABLE I.—THE COAGULATING POWER OF THE SAME RENNIN SAMPLE[1] ON DIFFERENT SAMPLES OF MILK.[2]

Day of July '29.	Milk sample No.	Time of coagulation.	Coagulating power.[3]	Day of July '29.	Milk sample No.	Time of coagulation.	Coagulating power.[3]
6	1	10.7	23,300	17	12	10.75[6]	23,200
7	2	9.2	27,200	18	13	12.80[7]	19,500
8	3	11.1	22,500	19	15	11.06[8]	22,600
9	4	10.9	22,900	20	16	12.06[9]	20,700
10	5	10.1	24,700	23	17	11.8[10]	21,100
11	6	8.6	29,000	24	18	9.00	27,800
12	7	11.10	22,500				
13	9	11.3	22,100	Maximum		12.8	39,000
14	10	6.4[4]	39,000	Minimum		6.4	19,500
16	11	11.40[5]	21,900	Average		10.5	23,800

SUB-NOTES TO TABLE I.

[1] Sample of Powdered Rennin No. 12 received at the Laboratory, 4–15–29.

[2] Whole Jersey milk, drawn about five o'clock A.M. and tested about ten o'clock A.M. of the same day. The temperature of the milk when delivered from the dairy ranged from 15°–18° C. and a temperature of from 13° C. to 18° C. was maintained thereafter. Babcock test, averaging about 5% butter-fat.

[3] N. F. V method used. Determinations made in a water-bath electrically controlled to

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