SCIENTIFIC SECTION

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

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

PART VII. CHANGES OCCURRING IN CRUDE ERGOT AND FLUIDEXTRACT OF ERGOT, U. S. P. X, DURING STORAGE.

BY MARVIN R. THOMPSON.*

The preceding articles of this series have confirmed that the value of ergot in medicine is due to two alkaloids, ergotamine and ergotoxine, which differ slightly in chemical composition and solubility, but which are pharmacologically identical.

The author is not convinced that each of these alkaloids actually exist in appreciable quantities as such in the crude drug, but believes that the process of isolation employed determines whether the product will be ergotamine (Stoll) or ergotoxine (Barger) which are equally active, or whether the product will be one of negligible activity, such as crystalline ergotinine (Tanret). The following evidence is offered to support this contention:

A single lot of crude ergot was mixed, assayed biologically by the Broom-Clark Method for alkaloidal activity, and divided into three equal portions. One portion, upon suitable extraction and chemical treatment, yielded an amorphous alkaloid which compared in chemical, physical and pharmaco-dynamic properties to ergotoxine $C_{35}H_{41}O_6N_5$ (50). The amount obtained was found, upon assay by the Broom-Clark Method, to represent approximately two-thirds of the total alkaloidal activity originally present in the crude drug. The second portion, when treated essentially according to the method of Stoll for the preparation of ergotamine, yielded an amount of crystalline ergotamine ($C_{33}H_{35}O_5N_5$) which, upon bio-assay, was found to represent approximately three-fourths of the total alkaloidal activity originally present in the crude drug. The third portion, by an entirely different chemical treatment, gave a copious yield of a needle-crystalline alkaloid which proved to be the ergotinine ($C_{35}H_{39}O_5N_5$) of Tanret (51). Upon careful examination by the usual biological methods, neither the marc, mother liquor nor the crystalline ergotinine exhibited significant alkaloidal activity.

It is obvious that the crude drug could not contain approximately 66 per cent of its total alkaloidal activity in the form of ergotoxine plus an additional 75 per cent of its total alkaloidal activity in the form of ergotamine. This illustration becomes all the more conclusive when account is given to the fact that the isolation of these labile alkaloids cannot be made quantitative. A loss of 20 to 25 per cent cannot be avoided when only relatively small quantities of the drug are involved.

The disappearance of significant activity from the marc, mother liquor, etc., of the portion from which the inactive crystalline ergotinine was prepared can only be explained by the hypothesis that the active alkaloid (either ergotamine or ergotoxine) was changed chemically into the inactive alkaloid because of the method of isolation employed. The slight differences in chemical composition and behavior of these three alkaloids have been reported by Barger (3, 6) (ergotamine

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

and ergotoxine) and Stoll (52) (ergotoxine and ergotamine). Barger found that ergotoxine (active) and crystalline ergotinine (inactive) could be changed from one to the other very easily since the crystalline alkaloid (ergotinine) is the anhydride of the amorphous (ergotoxine), as first suggested by Kraft.

Regardless of the form in which the alkaloidal activity exists in crude ergot or its galenicals, it is generally agreed that the therapeutic value of a preparation depends upon its effectiveness in producing paralysis of the motor endings or "myoneural junctions" of the sympathetic, and also the inhibitory fibers that are stimulated by epinephrine, as shown by Rothlin (31). Ergotoxine and ergotamine show practically equal activity with respect to this property, and therefore either may be regarded as being representative of the alkaloidal activity of ergot or its preparations. The Isolated Rabbit Uterus and the Cock's Comb Methods of assay measure this activity, the Rabbit Uterus Method being the more accurate of the two.

Crude ergot, or preparations thereof, must meet two requirements in order to be of medicinal value: *first*, a significant and defined amount of alkaloidal activity must be present, and *second*, this alkaloidal activity must be retained in a reasonably stable condition.

The first requirement can now be met, since sufficiently accurate methods for the estimation of alkaloidal activity are available, and since the U. S. P. X method for the preparation of Fluidextract of Ergot has been found to provide for the appearance in the preparation of practically all of the alkaloidal activity which was present in the parent drug. Before the second requirement can be met, however, the stability of the alkaloidal activity in crude ergot and in Fluidextract of Ergot must be known. Studies of this phase of the problem were therefore undertaken.

A. CRUDE ERGOT.

Previous Work on the Stability of Crude Ergot.

Forst (53) reported evidence showing that powdered ergot, when assayed by his chemical method, lost over 50 per cent of its alkaloid in six months. Burn and Ellis (54) examined a sample of whole ergot which was kept in a bottle and known to be at least fourteen years old, finding it to contain 0.075 per cent of alkaloid, which is approximately 150 per cent of the U. S. P. requirement, by the Isolated Rabbit Uterus Method of Broom and Clark. They (54) expressed a belief that ergot, if preserved in its original form, would lose very little of its activity over a period of many years.

Liptak (55) conducted experiments showing that ergot very gradually loses its activity and that, parallel with this loss in activity, there is an increase in the free fatty acid content of the fixed oil contained in the drug.

The U. S. P. and B. P. specifically require that crude ergot shall not be used if it is more than one year old, although there is no known method for determining the age of ergot by pharmacognostic, pharmaco-dynamic, or chemical examination. The author doubts the propriety of such a requirement since it has been found that ergot, if kept entire and in a dry condition for several years, continues to yield preparations and constituents entirely analogous in physiological activity to recently collected material. On the other hand, ergot stored in a damp condition rapidly undergoes objectionable changes, such as the development of mold, rancidity and fermentative or putrefactive decomposition with a resulting increase in non-specific or proteinogenous amine (histamine, etc.) formation.

EXPERIMENTAL.

The changes occurring in crude ergot due to age and storage conditions have been observed by making the following determinations in addition to pharmacognostic examinations from time to time:

1. Alkaloidal Activity.—A portion of the drug was exhausted of alkaloids by maceration and percolation with the acid-hydro-alcoholic menstruum of the U. S. P. The alkaloidal activity of the percolate was determined by the Isolated Rabbit Uterus Method (13) and checked by the Isolated Guinea-Pig Uterus Method for Alkaloidal Activity (39) and the Cock's Comb Method. Ergotamine tartrate was used as the standard for comparison.

2. Non-Specific Amine Activity.—This was carried out according to the method previously described for the determination of the non-specific amine activity of Crude Ergot (38), and checked by the usual pressor method to anæsthetized dogs. Histamine was used as the standard for comparison.

3. "Acid Number" of Fixed Oil.—The oil was extracted from a definite amount of the powdered drug by exhaustive percolation with petroleum benzine. The benzine was then driven off by exposing the percolate to a current of warm air until the weight became practically constant and the odor of benzine had disappeared. Five grams of the fixed oil was mixed in a glass-stoppered flask with 50 cc. of neutral alcohol, warmed to 50° C., and titrated with $N/10$ alkali with vigorous agitation, using phenolphthalein as an indicator. The persistence of the pink color for one minute with vigorous agitation was taken as the end-point. *The "Acid No.," in these results, is defined as the number of cubic centimeters of $N/10$ alkali necessary to neutralize the free fatty acid of ten Gm. of the fixed oil of ergot.*

CRUDE DRUG SAMPLES STUDIED.

Since Spanish and Russian Ergot constitute the two most important varieties, one lot of each was carefully selected. Both were of excellent appearance and characteristics and free from insect or mold infestation. They were also in a dry condition. The Russian lot was marked "R," and the Spanish was labeled "S."

Lot "R" was assayed and divided into three equal portions and labeled R_1, R_2 and R_3, respectively.

R_1 was ground and de-fatted with petroleum benzine, dried and stored in a loosely covered glass jar at room temperature. Twenty-two per cent of fixed oil was obtained. Due account for this loss in weight was made in subsequent determinations.

R_2 was stored in a paper bag in the crude or whole condition at room temperature in a dry place.

R_3 was stored in an air-tight glass jar, in the unground state, at room temperature.

TABLE VII.—CRUDE ERGOT "R."

Sample.	Date of exam. (plus or minus 10 days).	Known age of sample.	Alkaloidal content in terms of ergotamine tartrate,* per cent.	Non-specific amine content in terms of histamine,** per cent.	Acid no. of fixed oil.
	10-20-27	1927 crop	0.05–0.06	(−) 0.005	7.6
R_1	10-20-28	One year	0.05–0.06	(−) 0.005	..
	10-20-29	Two years	0.045–0.05	(−) 0.005	..
	10-20-27	1927 crop	0.05–0.06	(−) 0.005	7.6
R_2	10-20-28	One year	0.05–0.06	(−) 0.005	9.8
	10-20-29	Two years	0.045–0.05	(−) 0.005	12.4
	10-20-27	1927 crop	0.05–0.06	(−) 0.005	7.6
R_3	10-20-28	One year	0.045–0.055	(−) 0.005	11.7
	10-20-29	Two years	0.040–0.045	(−) 0.005	18.0

(−) Less than.

* Ergotamine Tartrate—The Sandoz Chemical Co.

** Histamine—The Pfanstiehl Chemical Co.

Lot "S" was assayed and divided into four equal portions and labeled S_1, S_2, S_3 and S_4, respectively.

S_1 was ground and stored in a loosely covered jar at room temperature.

S_2 was stored in its original from in a paper bag at room temperature in a dry place.

S_3 was stored in its original form in a sealed jar at room temperature.

S_4 was stored in its original form in a humidor container in a damp condition at room temperature.

All of these samples were examined at the end of one year, and again at the end of two years. The results obtained on Sample "R" are tabulated in Table VII, and those on Sample "S" are given in Table VIII.

TABLE VIII.—CRUDE ERGOT "S."

Sample.	Date of exam. (plus or minus 10 days).	Known age of sample.	Alkaloidal content in terms of ergotamine tartrate, per cent.	Non-specific amine content in terms of histamine, per cent.	Acid no. of fixed oil.
	10-20-27	1927 Crop	0.133	(−) 0.005	9.6
S_1	10-20-28	One year	0.09–0.10	(−) 0.005	64.8
	10-20-29	Two years	0.06–0.07	0.005	152.0
	10-20-27	1927 Crop	0.133	(−) 0.005	9.6
S_2	10-20-28	One year	0.125–0.130	0.005	17.4
	10-20-29	Two years	0.11–0.12	0.007	25.4
	10-20-27	1927 Crop	0.133	(−) 0.005	9.6
S_3	10-20-28	One year	0.12–0.13	(−) 0.005	35.6
	10-20-29	Two years	0.10–0.11	0.005	61.0
	10-20-27	1927 Crop	0.133	(−) 0.005	9.6
S_4	10-20-28	One year	0.06–0.07	0.06–0.07	37.9
	10-20-29	Two years	0.01–0.02	0.12–0.13	90.7

(−) Less than.

DISCUSSION OF RESULTS.

1. Alkaloidal Activity.—It is obvious from the results obtained from R_2, R_3, S_2 and S_3, that the loss of alkaloidal activity in whole ergot during storage over a reasonable period is not serious if the drug is kept in a dry condition. In addition to the experimental evidence to this effect contained in Tables VII and VIII, a sample of whole ergot which had been kept in a desiccator over calcium chloride for at least thirteen years at an unusually high temperature (near hot-water heating pipes) was found to have retained 0.06 per cent of alkaloid (approximately 120 per cent of U. S. P. potency) in terms of ergotamine. Since R_3 and S_3, which were stored in sealed jars, showed a very slightly greater loss in alkaloidal activity than R_2 and S_2, which were stored in bags, it is indicated that air-tight containers are certainly not necessary for storing crude ergot, and actually are to be avoided unless the drug is kept absolutely dry by a suitable dehydrating agent.

The results obtained from Sample R_1, which was ground and de-fatted before storing, and S_1, which was ground but not de-fatted, definitely show that the storage of *powdered* ergot results in an appreciable loss in alkaloidal activity unless the fixed oil is first removed. R_1 lost but a relatively small portion of its activity during two years, while S_1 lost approximately one-half of its alkaloidal activity in the same period.

S_4 was the only portion stored in the presence of moisture, or in a damp condition. This sample rapidly developed mold and a putrid, musty odor. At the end

of a year in this condition, half the activity had been lost, while in two years, only a relatively small amount of alkaloidal activity was retained.

2. *Non-Specific Amine Activity.*—All of the samples embraced in Tables VII and VIII were practically devoid of histamine or other active proteinogenous amines at the beginning of these studies. No significant changes in this respect occurred in the samples kept in a dry condition. Sample S_4, however, which was kept in a damp condition, rapidly developed non-specific amines which, for the most part, consisted of histamine.

3. *"Acid No." of Fixed Oil.*—All of the samples involved showed an increase in the free fatty acid content of their fixed oil as their respective ages increased. The "Acid Number" here described may be taken as an index of rancidity. The development of rancidity was found to be very slow if whole ergot is stored in bags in a dry place. The *powdered* drug S_1 developed rancidity to such an extent in two years that a heavy crystalline deposit of free fatty acid separated from the extracted fixed oil as the benzine was driven off. Tables VII and VIII show that the development of rancidity depends to a great extent upon storage conditions. The storage of whole ergot in open containers in a dry place is obviously preferable from this standpoint. Similarly, it is evident that *powdered* ergot must be de-fatted before storing to avoid objectionable changes in its composition.

In addition to the results contained in Tables VII and VIII, similar determinations were made upon 50 crude ergot imports for the purpose of ascertaining whether or not the "Acid Number" of the fixed oil was of value as a criterion in estimating the quality or age of the drug. The alkaloid and amine activity of these imports, together with the "Acid Number" of their fixed oil, is contained in Table IX.

TABLE IX.—THE RELATIONSHIP OF SPECIFIC ALKALOID AND NON-SPECIFIC AMINE ACTIVITY TO THE "ACID NUMBER" OF THE FIXED OIL OF IMPORTED CRUDE ERGOT.

Import P. C. No.	Consulate.	Variety.	Alkaloidal activity* in terms of U. S. P. Standard F. E., per cent.	Acid number of fixed oil.	Amine content in terms of histamine, per cent.
2001	Barcelona	Spanish	150	24.8	0.07
2003	Antwerp	?	150	14.3	0.02
2004	Hamburg	?	150	8.3	(−) 0.005
2005	Lisbon	Spanish	200	6.8	0.04
2015	Hamburg	Spanish	(=) 200	4.6	0.04
2016	Barcelona	?	100	30.8	0.08
2017	Marseilles	?	200	6.2	0.01
2019	Hamburg	Russian	50	23.7	0.05
2024	Moscow	Russian	75	27.0	0.04
2025	Moscow	Russian	80	21.1	(−) 0.005
2026	Hamburg	?	130	16.4	0.04
2031	Hamburg	?	120	8.6	0.07
2032	Brussels	?	175	4.5	0.02
2038	Hamburg	?	175	11.6	0.08
2039	Barcelona	Spanish	(=) 200	4.0	0.05
2049	Hamburg	?	175	6.5	0.08
2058	Hamburg	?	200	3.2	0.03
2063	Hamburg	?	(−) 50	13.7	0.01
2064	Hamburg	?	160	4.4	(−) 0.005
2065	Hamburg	?	160	5.2	——
2066	Barcelona	Spanish	(=) 200	6.0	0.06
2067	Hamburg	Russian	(=) 150	5.6	(−) 0.005

Import P. C. No.	Consulate.	Variety.	Alkaloidal activity* in terms of U. S. P. Standard F. E., per cent.	Acid number of fixed oil.	Amine content in terms of histamine, per cent.
2068	Dacon	?	200	6.4	0.07
2069	Hamburg	?	130	5.0	0.03
2074	Lisbon	Spanish	(=) 200	5.5	0.09
2076	Lisbon	Spanish	(=) 200	4.0	0.02
2077	Hamburg	?	200	4.8	(−) 0.005
2078	Hamburg	Russian	175	5.5	(−) 0.005
2079	Lisbon	Spanish	(=) 200	4.0	0.11
2080	Hamburg	?	130	7.7	(−) 0.005
2081	Hamburg	?	200	5.2	0.04
2083	Hamburg	?	200	5.5	0.04
2088	Hamburg	?	200	5.0	0.02
2094	Hamburg	?	200	6.0	(−) 0.005
2097	Hamburg	?	175	12.0	——
2098	Hamburg	?	(=) 200	4.0	——
2099	Moscow	Russian	100	12.0	(−) 0.005
2100	Brussels	?	(=) 200	4.4	——
2102	Hamburg	?	200	9.5	——
2104	Hamburg	?	200	12.0	——
2105	Danzig	?	100	9.5	(−) 0.005
2106	Danzig	?	133	10.5	0.01
2107	Budapest	?	100	6.5	——
2124	Lisbon	Spanish	100	42.5	0.12
2127	Vigo	?	100	24.6	0.07
2137	Hamburg	?	200	8.2	0.03
2141	Hamburg	?	(=) 200	4.2	——
2143	Hamburg	?	200	6.3	——
2151	Vigo	Spanish	(=) 200	2.6	——
2155	Hamburg	?	160	16.9	——

(=) Greater than.
(−) Less than.
* Cock's Comb Method, U. S. P.
? Origin or variety not stated.
—— Not determined.

Although the "Acid Number" of the oil of ergot was found to increase as the alkaloidal activity of the drug decreased during prolonged storage, it is evident from the results contained in Tables VII, VIII and IX that the "Acid Number" of the fixed oil conveys no indication as to the specific alkaloidal activity contained in the drug. An "Acid Number" above 15, however, does indicate a distinct rancid condition and suggests appreciable age or that the drug has been subjected to bad storage conditions.

The non-specific amine activity apparently is entirely independent of the alkaloidal activity or the "Acid Number," although a high "Acid Number" is often associated with a high non-specific amine content. If stored in a damp or moist condition in a warm place, the amine activity increases, presumably because of fermentation or putrefaction (see also Part IV (41)). The presence of mold often indicates a high histamine content, although the histamine content of some samples has been found to increase in the absence of mold. The presence of moisture is necessary for the development of either mold or non-specific amines, and can be effectively prevented by storage in a dry condition.

THE RELATION BETWEEN PHARMACOGNOSTIC CHARACTERISTICS AND THE QUALITY OF CRUDE ERGOT.

It is very difficult to express distinguishing pharmacognostic characteristics of different samples of ergot. This subject, therefore, must of necessity be treated more or less generally, and embraces conclusions drawn from the author's experience in the pharmacologic and pharmacognostic examination of over 200 samples of all varieties.

1. EXTERNAL APPEARANCE.

(*a*) *Color and Surface Markings.*—The surface color of the sclerotia varies from a light grayish purple or brown to a purplish black. The surface of the grains is usually fissured and furrowed longitudinally, although some samples are quite smooth. The various varieties show no significant differences which are of value in judging the amount of activity present.

(*b*) *Shape and Contour of Sclerotia.*—Longitudinal curvature and tapering or cross-section outline are not significant in determining the variety or quality of the drug.

(*c*) *Size of Sclerotia.*—Considerable variation size of grains is shown in all samples. The smallest grains of given samples usually have been found to show as great an amount of alkaloidal activity as the larger grains, although individual samples composed of the larger grains usually exhibit more alkaloidal activity than those composed of uniformly smaller sclerotia. Separation studies of single samples often lead to erroneous conclusions because of the samples being composed of mixtures of different lots. The grains of the Spanish or Portuguese varieties are usually larger than those of the Russian, Polish or German varieties, although occasional exceptions are found.

2. ODOR.

The odor of crude ergot varies with different samples. Some are almost odorless, some have a soapy, unpleasant odor, while others evolve a pleasant, fruity, aromatic odor. It is most evident in the ground condition. Samples containing the greatest amount of alkaloidal activity usually give off a very appreciable amount of the pleasant aromatic odor. Odorless samples, or those having the soapy odor with a suggestion of pungency or rancidity, are usually found to be relatively low in potency.

3. FRACTURE.

The fracture of the sclerotia has been found to be one of the most significant criteria as to the quality of ergot.

(*a*) *Ease and Shortness of Fracture.*—The most potent samples are composed of sclerotia which break easily with a short, corky fracture. Those having a tough, horny fracture are of poorer quality with respect to activity. The presence of an appreciably damp condition causes all varieties to show a somewhat rubbery fracture, thereby detracting from the significance of this test.

(*b*) *Texture of Fractured Surface.*—The smoothness or roughness of the fractured surface is not significant of the quality because of the influence exerted by the presence of moisture.

(*c*) *Color of Fractured Surface.*—The best quality is denoted by a white or yellowish white color. Appreciable amounts of bluish, purplish or brownish tint is indicative of poorer quality from the standpoint of activity. These tints usually increase in intensity with the age of the sample.

4. GRINDING CHARACTERISTICS.

(*a*) *Ease of Grinding.*—This criterion of quality is closely related to the ease of fracture. Samples containing a high degree of potency are reduced to a powder much more easily than those of poor quality.

(*b*) *Texture of Powdered Drug.*—A soft, mealy texture of the coarsely powdered drug is to be preferred. The fragments of ground ergot of poor quality are usually sharp and angular, giving the powder a rough texture. The difference is best detected by rubbing the powder between the fingers.

(*c*) *Color of the Ground Ergot.*—The color of different samples of powdered ergot varies from a light purplish gray to a grayish brown. A predominance of brown is to be preferred. The purplish gray color is characteristic of the poorer quality samples of Russian and Polish Ergot.

5. FIXED OIL.

(*a*) *Quantity.*—The quantity of fixed oil can be determined by de-fatting in the usual manner, and driving off the benzine by distillation or by exposing to a current of air until the weight becomes constant. The amount contained in ergot varies from 10 to 35 per cent. The best quality is usually indicated by a high fixed oil content, although the age of the sample must also be considered.

(*b*) *Color.*—The color of the extracted oil varies from a very light amber to a dark amber. This color has not been found to be a significant indication of the potency of the drug, although a deep amber color has been found to indicate appreciable rancidity (a high "Acid Number" or "Free Fatty Acid" content).

6. EXTRACTIVES OBTAINED DURING PREPARATION OF FLUIDEXTRACT, U. S. P.

Although the active principles of ergot have been found to represent but a small portion of the extractable material contained in ergot (48), which imparts the dark color to fluidextracts, the color of the percolate is usually quite significant. Samples containing relatively low potency usually yield a percolate which is relatively transparent and which shows a predominance of red coloration. The more potent samples yield a percolate in which a rich brown color predominates instead of red, and which is comparatively much deeper in color and of greater "total solid" content. These characteristics have been found to be true only at the time of percolation or preparation of the fluidextract. Changes in the color rapidly take place during storage, with a result that all types and qualities have a tendency to assume similar appearances.

7. MOLD AND INSECT INFESTATION.

(*a*) *Insect Infestation.*—The author has examined samples of ergot from time to time which had been literally alive with mites and lice for many months. No significant changes in the activity of fluidextracts of these insect-infested samples were observed. Nevertheless, insect infestation is decidedly objectionable from other obvious points of view. The use of small amounts of carbon disulphide naphthalene or carbon tetrachloride can be made to prevent this condition without appreciably interfering with the activity of the drug.

(*b*) *Mold.*—Moldy conditions are equally objectionable. The presence of moisture is essential to mold development. Whether the mold is present or not, moisture increases the rate of deterioration of the alkaloidal activity and has a tendency to cause an increase in proteinogenous amine formation, especially in a warm temperature.

8. CHOICE OF VARIETIES.

Much dispute is found in the earlier literature with respect to this point. The most important varieties of Ergot of Rye include Spanish, Portuguese, Russian and Polish. Different samples of Spanish and Portuguese ergot have been found to yield alkaloidal activity ranging from 0.05 to 0.30 per cent in terms of ergotamine or ergotoxine, while the alkaloidal activity of Russian and Polish varieties examined were found to vary from 0.02 to 0.10 per cent in terms of ergotamine or ergotoxine.

Since the therapeutic value of ergot depends upon its alkaloidal activity, the Spanish and Portuguese varieties are to be preferred. From the qualitative standpoint of physiological activity, however, the different varieties have exhibited no distinguishing characteristics. *Therefore, if Russian or Polish samples are found to contain a satisfactory amount of alkaloidal activity by*

the usual biological tests, their resulting standardized fluidextracts are equal in quality to those prepared from other varieties.

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

Previous Work on the Stability of Fluidextract of Ergot.

It has been known for many years that ergot preparations lose their activity upon standing. The rate and factors which influence the rate of deterioration constitute a much disputed subject.

The U. S. P. requires that the Standard Fluidextract of Ergot be aged for six months prior to final standardization because a thought holds sway among investigators in this country that the most rapid deterioration of potency takes place during this period, after which a greater degree of stability is manifest.

Prybill and Maurer (56) examined liquid extracts prepared by five different pharmacopœial processes, in which they found that only the U. S. P. X and the D. A. B. VI extracts exhibited a significant amount of alkaloidal activity. Turning to the stability of these extracts, they found that the alkaloidal activity of the German Pharmacopœial preparations deteriorated at a more rapid rate than that of the U. S. P. X Fluidextract, but that even the U. S. P. preparations lost half of their activity in forty-eight weeks.

Wokes (57) reported extensive research showing that all types of the usual liquid and semi-solid extracts of ergot rapidly lose their activity on keeping, concluding that the " . . . rate of deterioration is influenced by the temperature at which the liquid extract is stored, being about twice as rapid at room temperature as in the ice chest, and still more rapid in the incubator at 37° C. Even under the best conditions, however, half the activity is lost in two or three months . . . . ," and that " . . . . concentrated (soft) extracts of ergot are rather more stable. When stored in the ice chest they retain half their activity for four to nine months."

The author, in a preceding article (42), showed that deterioration of the alkaloidal activity of Fluidextract of Ergot, U. S. P. X, was not nearly as rapid as is stated by Wokes *provided that the preparation is protected from excessive exposure to air.* Results obtained upon ten samples of fluidextracts at various intervals for nine months showed that while the rate of deterioration is somewhat more rapid during the first two or three months after preparation, approximately two-thirds of the activity was retained at the end of nine months in most instances. It will be noted that these samples were kept in completely filled, tightly sealed bottles in a cool place protected from light. Portions were drawn for assay by inserting a fine hypodermic needle through the stopper, and immediately re-coating the seal with wax.

Stoll and Rothlin (9), in 1927, reported evidence showing that exposure to air or oxygen quickly destroyed the activity of the ergot alkaloids.

Swanson (34) recently reported evidence indicating that the hydrogen-ion concentration factor influenced the rate of deterioration of ergot activity. A lack of acidity was found to result in an increase in the rate of deterioration.

EXPERIMENTAL.

Ten 500-Gm. portions of crude ergot were carefully selected from recent importations. These portions were mixed, ground, de-fatted and converted into five liters of Fluidextract of Ergot by the U. S. P. X process. This preparation was biologically assayed for alkaloidal activity

by the U. S. P. Cock's Comb Method, and found to contain between 0.1 and 0.12 per cent of alkaloid in terms of ergotamine tartrate (Sandoz Chemical Co.). Pressor and Isolated Guinea-Pig Uterus experiments (38) showed that practically no non-specific amines were present.

This fluidextract was divided into suitable portions and stored as indicated below.

B (1) was stored in a clear-glass, completely filled, cork-stoppered bottle at room temperature on a laboratory shelf. The cork was removed at three-month intervals for the time necessary to draw a portion for assay.

B (2) was stored in a completely filled, cork-stoppered, amber-colored bottle and was subjected to the same conditions as B (1).

B (3) was kept in a half-filled, amber-colored, cork-stoppered bottle under the same conditions as B (1) and B (2).

B (4) was kept in an amber-colored, half-filled, cork-stoppered bottle in a refrigerator. The cork was removed at three-month intervals only for the time necessary to remove portions for assay.

B (5) was kept in a completely filled amber-colored bottle under the same conditions as B (4).

B (6) was kept exposed to air in an unstoppered, amber-colored bottle under laboratory conditions. Evaporation was corrected for by bringing to the original volume with diluted alcohol.

B (7) was kept exposed to air in an unstoppered bottle, in a refrigerator.

B (8) was sealed in hard-glass 5-cc. ampuls *in vacuo* and kept at refrigeration temperature.

The bottles which were completely filled were kept so during the entire time of the experimentation by the addition of glass beads at the time of removal of portions for assay.

Most of the assays were carried out by the Cock's Comb Method, which has been shown to be reasonably accurate in measuring the alkaloidal activity in the absence of high proportions of non-specific amines (42). Occasional check determinations were made by the Isolated Rabbit Uterus Method (28) and the Isolated Guinea-Pig Uterus Method for alkaloidal activity (39), especially during the last two 3- or 4-month intervals for each preparation. Ergotamine tartrate was used as the standard throughout the entire experiment, because this crystalline alkaloid was known to be constant in composition and potency.

The potency of all eight of the samples was determined at the intervals indicated, until they were found to be practically inert. The results are given in Table X, in which the potency of the preparations is expressed in per cent, taking 0.50 mg. of ergotamine tartrate per cc. as 100 per cent.

TABLE X.—THE DETERIORATION OF FLUIDEXTRACT OF ERGOT UNDER VARIOUS CONDITIONS OF STORAGE.

	Oct. 20, 1927.	Per Cent Alkaloidal Activity* at Age of:						
F. E. no.	Freshly prepared.	3 months.	6 months.	9 months.	12 months.	16 months.	20 months.	24 months.
B (1)	200–250	150–170	125–150	80–90	50–60	20–30	Inert**	———
B (2)	200–250	150–170	125–150	90–110	60–80	40–50	20–25	Inert**
B (3)	200–250	100–125	60–75	20–30	Inert**	———	———	———
B (4)	200–250	130–150	90–110	50–70	40–50	25–35	Inert**	———
B (5)	200–250	175–200	160–180	150–170	130–160	125–135	100–125	70–90
B (6)	200–250	Inert**	———	———	———	———	———	———
B (7)	200–250	50–75	Inert**	———	———	———	———	———
B (8)	200–250	———	200–225	———	175–210	———	———	160–180

* 0.05 per cent Ergotamine Tartrate taken as 100%.

** "Inert" indicates a potency of less than 20%.

DISCUSSION OF RESULTS OF TABLE X.

The results obtained conclusively show:

(*1*) That exposure to air is the most important of the factors which hasten the deterioration of the specific alkaloidal activity of Fluidextract of Ergot;

(*2*) That storing in a refrigerator decreases the rate of oxidation and subsequent destruction of the ergot alkaloids;

(*3*) That the rate of deterioration is somewhat more rapid during the first three months, after which a slower and more constant rate is shown; and

(*4*) That even if the preparation is kept in partially evacuated ampuls, a slight, but definite loss in activity cannot be avoided.

This evidence immediately suggests that the most satisfactory fluidextract may be marketed by preparing as described in the U. S. P. X process, but concentrating the exhaust percolate (obtained after the reserve portion) *in vacuo* at a low temperature. The finished preparation should then be aged for a period of three months in completely filled glass or glass-lined containers in a refrigerator or cold room. At this point, the preparation must be assayed for alkaloidal activity or content, the hydrogen-ion concentration must be determined, and the preparation standardized to the correct potency as near to the time of shipment as possible. If dilution is necessary, the diluent must be composed of diluted alcohol, acidified with hydrochloric acid to the same hydrogen-ion concentration as was found in the preparation.

Fluidextract of Ergot must be stored in well-filled, tightly-stoppered, glass containers in a refrigerator. Even under these conditions the stability is not as great as that indicated by B (5) of Table X, unless the containers are kept completely filled at all times, or any space in the bottle above the liquid is occupied by an inert gas such as nitrogen or carbon dioxide. Since the latter condition cannot be provided commercially, or by the dispensing pharmacist, the activity of Fluidextract of Ergot which has attained an age of more than one year from the date of assay and standardization will be far too uncertain to be of clinical value. If kept in ampuls, sealed *in vacuo*, the greater part of the activity will be retained for over two years.

PART VIII. A CONSIDERATION OF BIO-ASSAY STANDARDS FOR ERGOT AND ITS PREPARATIONS.

An ideal standard for the biological assay of crude ergot or its preparations must meet two fundamental requirements. *First*, its pharmaco-dynamic activity must be representative of the clinically desirable activity of ergot; and, *second*, it must be constant in activity and composition.

It has been demonstrated both pharmacologically and clinically that the desirable activity of ergot is due to the specific alkaloids. Several reasonably accurate biological methods are now available for the estimation of this alkaloidal activity (see preceding articles of this series), besides at least one chemical (colorimetric) method, which has been reported by Van Urk (36). All of these methods, whether biological or colorimetric, require the use of a standard for comparison.

THE RELATIVE APPLICABILITY OF STANDARDS NOW IN USE.

1. The Official Standard of the U. S. P.

The U. S. P., tenth revision requires the use of a fluidextract, prepared by the official process therein described as the bio-assay standard for crude ergot and Fluidextract of Ergot.

To avoid individual potency variation of different lots of crude ergot the U. S. P. requires that this Standard Fluidextract shall be a composite, representing at least ten different lots of ergot conforming to the official botanical description. This fluidextract is to be aged for at least six months, then standardized by the official Cock's Comb Method, and preserved in vacuum.

The experimental studies of this investigation have resulted in conclusive evidence to the effect that the procedure specified for the preparation of the U. S. P. Standard Fluidextract results in one which owes its activity to a significant specific alkaloidal content, and that no detectable amount of histamine or other non-specific amines are present. In other words, this standard meets the first one of the two fundamental requirements enumerated in the first paragraph of this article. Just how completely this standard satisfies the second requirement of an ideal standard, *i. e.*, that of constancy and stability, will next be considered.

Since individual lots of crude ergot conforming to the U. S. P. botanical description show enormous differences in alkaloidal potency, it is a foregone conclusion that it would be impossible to prepare different lots of fluidextract which would be identical in potency. Therefore, the U. S. P. directs that, in the preparation of the Standard, the aged fluidextract shall be standardized by the official Cock's Comb Method before being preserved in air-tight containers *in vacuo*.

Gittinger and Munch (15), Pattee and Nelson (28), and the present writer (42) have shown that even a reasonable degree of accuracy in the estimation of the alkaloidal activity of Fluidextract of Ergot by the Cock's Comb Method cannot be attained unless the individual reactions of the cockerels to a preparation of known potency had previously been determined. Since no ergot preparation of known potency was available at the time of the preparation of the first U. S. P. Standard Fluidextract, the indvidual "thresholds" (15) of the cockerels involved was not known. Therefore, the preparation could be standardized only by adjusting the potency such that 0.5 cc. per Kg. would produce a satisfactory bluing in the *majority* of a series of cockerels of U. S. P. specifications. In the light of present knowledge, such procedure could result in only an approximate estimate of the potency, indicating that the actual value was between 0.04 and 0.06 per cent of alkaloid in terms of either ergotamine or ergotoxine. It is obvious that greater accuracy was hardly necessary for the first standard since the adopted potency factor was purely arbitrary, and was intended to represent that of a good quality, therapeutically active Fluidextract of Ergot. The individual "threshold doses" of cockerels could then be determined, and the task of adjusting the potency of succeeding lots of Standard would present no difficulties, *provided that the first lot had been absolutely stable after being sealed in ampuls in vacuo.*

The author, in the preceding article (49), has shown that fluidextract is not absolutely stable even when kept in partially evacuated ampuls, although the loss in potency over a reasonable period (one year or less) is not great.

The specific ergot alkaloids ergotamine and ergotoxine have been available in their pure state for several years. Since their appearance, some very definite information has been obtained with respect to the strength of the U. S. P. Standard. Pattee and Nelson (28) found U. S. P. Standard Fluidextract, Lot No. 635,[1] to contain 0.045 per cent of alkaloid in terms of ergotamine base, by both the Broom-Clark Rabbit Uterus Method and the Cock's Comb Method. This lot (635) which is now over four years old, has been very carefully assayed (February 1930) by the author, using the same methods, and found to contain less than 0.02 per cent alkaloid in terms of ergotamine base. Lot No. 636[1] of the Standard Fluidextract replaced Lot No. 635 over two years ago. Lot No. 636 has been carefully and recently assayed by the author, by Dr. J. H. Burn of the Pharmaceutical Society of Great Britain, and by Dr. M. I. Smith of the Hygienic Laboratories of the U. S. Public Health Service, all agreeing that Lot No. 636 contained certainly not more than 0.03 per cent of ergot alkaloids in terms of either ergotamine or ergotoxine, while many assays have indicated that the actual alkaloid content does not exceed 0.025 per cent.

The results obtained in this investigation have conclusively shown that a fluidextract containing only 0.03 per cent of ergot alkaloids will not consistently produce a satisfactory cock's comb reaction in doses of 0.5 cc. per Kg. unless the susceptibility of birds has been increased due to frequent usage (41, 42). Since the standard did produce the correct reaction at the time it was sealed in ampuls, it can only be concluded that a loss in potency has occurred. The values given for Lots 635 and 636, together with the results reported in a preceding article (49) confirm this

[1] Lot number applied by the U. S. Department of Agriculture.

conclusion, and indicate that both Lot Nos. 635 and 636 contained approximately 0.05 per cent of ergot alkaloids at the time they were ampuled.

At the present time Standard Fluidextract of Ergot No. 2160[1] has replaced No. 636. This new lot of standard has been found to contain 0.05 per cent of ergot alkaloids in terms of ergotamine base by the use of the official Cock's Comb Method (15), the Broom-Clark Rabbit Uterus Method (28), and the Isolated Guinea-Pig Uterus Method for the estimation of ergot alkaloids (39).

It is evident that the U. S. P. Standard for the bio-assay of ergot and Fluidextract of Ergot does not fully meet the second fundamental requirement stated at the beginning of this article, although by preparing a new lot of this standard every eight or ten months, the very slight change in potency would not constitute a great objection.

2. The Use of Ergotamine or Ergotoxine as a Standard.

Both of these specific alkaloids have been found to be entirely representative of the desirable activity of ergot (see preceding articles of this series) by all of the known methods of study. Therefore, either one of these alkaloids meet the first requirement of an ideal standard.

As to constancy and stability, both apparently are superior to the present U. S. P. Standard Fluidextract. Ergotoxine is now available as the ethanesulphonate and phosphate, and ergotamine as the tartrate and the methanesulphonate. The author has no information concerning the stability of the ergotoxine salts, but has found that three ampuls each of crystalline ergotamine tartrate and methanesulphonate which were known to be at least five years old have remained identical in potency, solubility and appearance with samples recently obtained, thereby indicating practically absolute stability during five years.

The potencies of ergotamine and ergotoxine are identical for all practical purposes. Pattee and Nelson (28) and Swanson (34) agree that ergotoxine is slightly more active than ergotamine, but that, even by the Broom-Clark Method, a great number of determinations must be made before the slight difference in potency becomes evident. The actual difference, therefore, is practically no greater than that of experimental error in the Broom-Clark Method, and cannot be conclusively demonstrated by the less accurate Cock's Comb Method.

The solubilities of the salts of these two alkaloids differ appreciably. The ergotoxine salts are very difficultly soluble, yielding a colloidal suspension with water. A clear, true solution results only when an appreciable amount of dilute acid (hydrochloric, phosphoric, etc.) or alcohol is added. The salts of ergotamine are definitely crystalline and yield clear aqueous solutions readily and without trituration if a mere trace of hydrochloric acid is added. Solutions of both alkaloids are prone to deterioration, the rate and influencing factors of which are identical to those described in a preceding article for Fluidextract of Ergot (49).

The use of either ergotamine or ergotoxine as a bio-assay standard for ergot would, therefore, present no advantages over the present standard unless they were employed as the dry substances. As such, either of these alkaloids fully meet all of the requirements of a suitable standard, since they are representative of the therapeutic activity of ergot, they are definite chemical compounds of known composition, they are stable in the dry condition and fresh solutions of known potency can be easily prepared.

The Pharmaceutical Society of Great Britain has adopted ergotoxine phosphate as the standard for ergot preparations. This substance is undoubtedly satisfactory in this connection, but it is believed that ergotamine tartrate offers distinct advantages over ergotoxine phosphate or ethanesulphonate in that it is definitely crystalline and is more easily placed in perfect aqueous solution.

THE SELECTION OF A SUITABLE POTENCY REQUIREMENT FOR FLUIDEXTRACT OF ERGOT, U. S. P.

The Pharmaceutical Society of Great Britain has recently adopted a potency requirement of 0.05 per cent ergotoxine phosphate for Liquid Extracts of Ergot, and

does not grant a certificate to any product which does not meet this standard (58). Patee and Nelson (28) expressed a belief that this value constitutes a suitable potency for Fluidextract of Ergot from the standpoint of the clinician as well as the manufacturer. Bourne and Burn (59) found that the effective hypodermic dose of ergotamine or ergotoxine in obstetrics is between 0.5 and 1.0 mg. Since oral doses usually must be somewhat larger because of the absorption factor it is believed that at least 1.0 mg. of alkaloid should be contained in a dose of fluidextract. A 0.05 per cent alkaloid content corresponds to 0.5 mg. per cc. Therefore, this value provides for an alkaloid content of 1.0 mg. in the U. S. P. dose of Fluidextract of Ergot.

As a result of a thorough review of ergot literature and because of the results obtained in this investigation, the author agrees that an alkaloid content of 0.05 per cent, in terms of either ergotamine or ergotoxine base, in Fluidextract of Ergot provides for a therapeutically active preparation which will be satisfactory to the physician. Because of his experience in biologically testing practically all lots of crude ergot imported during the past two years and in testing fluidextracts marketed by practically every manufacturer in this country, the author is confident that Fluidextract of Ergot of this potency can be satisfactorily prepared from the crude ergot now available.

(*To be continued*)

A PHARMACEUTICAL STUDY OF HYDRASTIS CANADENSIS.

BY RUBY HIROSE AND H. A. LANGENHAN.

(*Continued from p. 353, April Issue.*)

ASSAY AND PURITY RUBRIC.

The 1900 U. S. Pharmacopœia (1) was the first to introduce either a purity rubric or an assay. The rubric read, "not less than 2.5 per cent of Hydrastine." The 1910 revision (2) introduced a limit of leaves, stems and foreign matter and changed the alkaloidal requirement to, "not less than 2.5 per cent of ether-soluble alkaloids." The revision of 1920 (3) added to this, "and not more than 3 per cent of acid-insoluble ash."

The assay method introduced into the revision of 1900 (4) consisted of macerating 15 Gm. of Hydrastis No. 60 powder with 150 cc. of ether and 5 cc. of ammonia water for one-half hour; then adding 15 cc. of water to cause the drug to agglutinate, and decanting 100 cc. of the supernatant liquid. The ethereal liquid is extracted with several portions of aqueous sulphuric acid solution; the combined acid solutions made alkaline with ammonia water and extracted with ether. The combined ether extractions evaporated to a constant weight at 100° C. The revision of 1910 followed the same procedure except that the quantity of drug used is 10 Gm., instead of 15 Gm.; and 100 cc. of ether is added; then decanting 50 cc. This procedure is also included in the 1920 revision of the U. S. P.

With no distinct change in the assay process as given in the revisions of 1900 and 1910, it may be assumed that the results obtained were "ether-soluble alkaloids" and not Hydrastine as specified by the 1900 revision.