center, convulsions and death. In Fig. 4, at B, 1 cc. of the weaker mercuric cyanide counterfeit for mercurochrome was injected and, it will be noted, produced a distinct and marked stimulation of the respiration, indicated by the wider excursion of the tambour. At Mer., 2 cc. of the stronger mercuric cyanide antiseptic nostrum were injected. The respiration was stimulated for a second or two and was then paralyzed; the animal went into convulsions and died within three minutes. The amount of substance required to demonstrate the characteristic effect on the respiration in these experiments was a very small one. It may be well to add that administration of a small quantity of the second preparation to a rabbit by stomach also gave a characteristic picture of cyanide poisoning, 10 cc. producing death within ten minutes. Curiously enough, while both of the fake "mercurochrome" preparations contained a dangerous poison mixed with inert dye, a bacteriological examination of both specimens, made by Mr. Eric Drake, revealed that the first specimen was not antiseptic at all while the second was only very slightly so.

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

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

PART IV. STUDY OF AQUEOUS EXTRACTS OF ERGOT.

BY MARVIN R. THOMPSON.[1]

The most widely used preparations of Ergot may be conveniently divided into two classes, depending upon the menstruum employed in the extraction processes, as follows: (*a*) Those involving the use of an aqueous menstruum for the extraction, such as Extractum Ergotæ Aquosum, N. F. (23), (commonly known as "Ergotin" or "Ergotine"), Extractum Ergotæ, and Extractum Ergotæ Liquidum of the B. P. (40); and (*b*) those involving the use of hydroalcoholic or acid-hydroalcoholic menstrua, as exemplified by Fluidextractum Ergotæ of the U. S. P. (24). Most proprietory preparations of Ergot also fall into one or the other of these two classes (see "New and Non-official Remedies," 1928 and 1929).

The aqueous preparations only are considered in this report. A study of the preparations of the second class will be reported in another paper of this series.

PREPARATION OF AQUEOUS EXTRACTS OF ERGOT.

The pharmaceutical procedure for the manufacture of Aqueous Extract of Ergot, N. F. (23) and that for the manufacture of Extract of Ergot and Liquid Extract of Ergot, B. P. (40) are essentially the same. In each case, the drug is extracted with an aqueous menstruum, the aqueous extract is concentrated at a moderate temperature, and supposedly inert material is precipitated by the addition of alcohol and filtered. The concentration of Liquid Extract of Ergot, B. P., is adjusted so that 1 cc. represents the water-soluble physiologically active principles of 1 gram of crude ergot. In the preparation of both the B. P. and N. F. semi-solid Extract of Ergot the aqueous extract must be further concentrated to a semi-solid or pilular consistence. Neither the N. F. nor the B. P. require the preparations to be standardized in any way.

The N. F. and B. P. differ slightly as to the aqueous menstruum employed in their preparations. The N. F. specifies chloroform water while the B. P. specifies water as the extraction men-

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struum. The small amount of chloroform in the menstruum of the N. F. preparation is not sufficient to alter the solvent properties of the water, but is intended to inhibit fermentation or putrefaction during the extraction process. Theoretically, then, both processes should yield similar products with respect to nature and amount of activity, as the small amount of chloroform present in the N. F. menstruum is expelled by evaporation during the concentration of the extract.

Although these aqueous Extracts of Ergot have received considerable attention in the literature, most of the studies reported have been incomplete. The pharmaco-dynamically active constituents of ergot have been discussed (37). Briefly, they consist of a series of specific alkaloids, of which ergotoxine and ergotamine are the most important; and a series of non-specific amines, such as histamine, tyramine and cholines. Several investigators (16 13, 19,) have found that aqueous extracts of ergot owe their pharmaco-dynamic activity to the non-specific amines of the drug and that preparations of this type are practically devoid of the specific alkaloids. Since, in the light of present-day opinion, the alkaloids are the specific and desirable active constituents of ergot from the clinical standpoint (1, 19, 28, 34), it might be concluded that Extracts of Ergot prepared by extraction with water are valueless with respect to ergot therapy.

A study of these aqueous Extracts of Ergot was undertaken for the purpose of determining the nature of their activity and the factors which influence the activity of ergot preparations of this type. The experimental studies embraced aqueous extracts of ergot of the following types, the numbers corresponding to those in Table II:

(*1*) Extractum Ergotæ, B. P. Method:
- (*a*) Allowed to ferment during aqueous extraction.
- (*b*) Not permitted to ferment during aqueous extraction.

(*2*) Extractum Ergotæ Liquidum, B. P. Method:
- (*a*) Allowed to ferment during aqueous extraction.
- (*b*) Not permitted to ferment during aqueous extraction.

(*3*) Extractum Ergotæ Aquosum, N. F. Method:
- (*a*) N. F. method in detail; evaporated to *pilular* consistence.
- (*b*) N. F. method, except that the concentration was adjusted by evaporation to a point such that 1 cc. of the chloroform-water alcohol-precipitated extract represented 1 gram of crude ergot.
- (*c*) Same as 3 (*b*), except that the ground drug was de-fatted with petroleum benzine before proceeding with the chloroform water extraction.

(*4*) *Alkaline Aqueous Extract of Ergot, Special:*

Prepared by de-fatting with petroleum benzine and exhausting the drug by percolation with a 3 per cent solution of sodium bicarbonate in water at a temperature below 10° C. to prevent fermentation. Twenty per cent of alcohol was added to the finished product.

(*5*) *Acid Aqueous Extract of Ergot, Special:*

Prepared as No. 4, except that the extraction menstruum consisted of 0.2 per cent hydrochloric acid U. S. P. in water. Twenty per cent alcohol was added to the finished product to prevent fermentation.

Up to the present time, available chemical methods have not proven satisfactory in accurately determining the nature of ergot preparations. Several biological methods have been found to yield reasonably accurate qualitative and quantitative information on the activity of the various constituents of ergot. Hence, the following biological methods were used in this study:

(*a*) *Cock's Comb Method,* official in the U. S. P. X, for the assay of Fluidextract of Ergot. The method as employed has been described in detail by Edmunds and Hale (30), by Gittinger and Munch (15), and by Pattee and Nelson (28). It is reputed to measure the alkaloidal activity of the ergot preparation. Sollmann (14) states that the amines may influence results by this method, although others (28, 34) have found it to yield results comparable to those obtained by the Broom-Clark Isolated Rabbit Uterus Method for the estimation of the alkaloidal content of Ergot preparations. The cockerels used met the specifications of the U. S. P. They were those used in the routine assay of ergot preparations in the Pharmaco-

logical Laboratory. Their individual reactions to the U. S. P. Standard Fluidextract of Ergot were determined every three months to correct for individual changes in susceptibility and tolerance from time to time, as recommended by Gittinger and Munch (15) and Pattee and Nelson (28). Two groups of birds were employed. Those in one group, designated "old cockerels" in the tables, had been standardized and used every two weeks for 6 months or more in testing ergot preparations. Those in the other group, designated "new cockerels" in the tables, were new birds that had never undergone ergot testing, aside from two or three injections of Standard Ergot at 2-week intervals for the purpose of establishing their threshold reactions (15). The two groups were used to determine whether or not continued usage played any part in the pharmacodynamic response of the birds to various active constituents of ergot.

(*b*) *Histamine-Ergot Alkaloid Inhibition Method,* involving the use of the isolated guinea-pig uterus. This method, described in a preceding article (39) serves to measure the alkaloidal activity of the preparation by the ability of definite concentrations of ergot alkaloids to inhibit the response of the guinea-pig uterus to constant doses of histamine.

(*c*) *Broom-Clark Isolated Rabbit Uterus Method.*—The details of this method are described by Pattee and Nelson (28) and are similar to those described by Broom and Clark (13) except that the modification proposed by Pattee and Nelson, and found by the authors to obviate possible amine interference, was used. The modified method was used because aqueous Extracts of Ergot were found to contain a higher proportion of amines to alkaloids than alcoholic extracts. This method is reputed to measure the alkaloidal activity of the ergot preparations (13, 28, 19, 31, 33, 34) irrespective of the amine content.

(*d*) *Isolated Guinea-Pig Uterus Method* for the determination of the amine activity of ergot preparations. This method, already described in detail (38) provides for the estimation of the resultant amine activity expressed in terms of histamine. Alkaloidal interference is obviated by observing the details described. All of the amines identified with ergot stimulate the described tissue to contraction. Therefore, the method serves to estimate the resultant effect of all of the amines present, but does not serve to differentiate between or indicate which of the amines are present and responsible for the observed uterine activity. Parallel pressor studies on cats or dogs are of great assistance in determining which of the two most important amines (histamine and tyramine) predominates in mixtures, as both exhibit decidedly differing activity upon the blood pressure of the usual test animals. Nelson and Pattee (1) state that although no positive statement is available as to which of these amines predominate, it is generally agreed that most of the amine activity may be ascribed to histamine. The results of these studies tend to confirm this statement.

(*e*) *Pressor Method to Anesthetized Dogs.*—The usual technique was followed in carrying out this method. Male or female dogs weighing approximately 10 kilograms were anesthetized by the intraperitoneal injection of 125 to 150 milligrams per kilogram of sodium luminal. Both vagi were severed and artificial respiration was administered. The blood pressure was recorded from the carotid artery and the drug preparations were introduced by way of the canulized femoral vein in the usual manner. This method has been applied qualitatively only, because it was practically impossible to obtain accurate quantitative information owing to susceptibility variations in dogs and the difference in the pressor activity of the various amines and alkaloids of ergot. The blood pressure studies are therefore considered apart from those involving other methods.

CRUDE DRUG SAMPLES USED IN PREPARATION OF EXTRACTS.

A rather large number of samples of crude ergot of several varieties have been used in this work. The experimental results obtained on ten of these samples are included in Tables I and II. It is believed that these results are typical for the

TABLE I.—CRUDE DRUG SAMPLES OBTAINED FROM IMPORTED ERGOT.

Crude drug no.	Commercial source.	U. S. P. X POTENCY* OF CRUDE DRUG. Old cockerels. *Per cent.*	U. S. P. X POTENCY* OF CRUDE DRUG. New cockerels. *Per cent.*	TOTAL ALKALOIDS OF CRUDE DRUG, CALCULATED AS ERGOTAMINE BASE. Broom-Clark Isolated Rabbit Uterus Method (28). *Per cent.*	TOTAL ALKALOIDS OF CRUDE DRUG, CALCULATED AS ERGOTAMINE BASE. Isolated Guinea-Pig Uterus Method for alkaloids (39). *Per cent.*	Total amines of crude drug, calculated as histamine, by Isolated Guinea-Pig Uterus Method for amines (38). *Per cent.*
1	Russian	130–150	160–170	0.05	0.04	0.035
2	Polish	175–200	175–200	0.067	0.06	0.015
3	Russian	25–80	100	0.036	0.03	0.045
4	Russian	200–225	200–225	0.075	0.07	0.033
5	Spanish	300–450	500	0.150	0.15	0.052
6	Spanish	400–450	400–450	0.133	0.12	0.027
7	Portuguese	250–275	250–275	0.080	0.08	0.012
8	Portuguese	150–250	350–400	0.120	0.11	0.087
9	Spanish	100–350	200–300	0.125	0.12	0.125
10	Spanish	100–350	200–300	0.133	0.13	0.150

* Potency in terms of U. S. P. Standard Fluidextract of Ergot.

general run of crude ergot imported into the United States. Several samples have been found to be practically devoid of amine activity by pressor and uterine methods (38), but significant amounts of the alkaloids were invariably present. Most of the samples examined contained appreciable amounts of amines, regardless of alkaloidal content, variety or origin. A few investigators in this country have recently contended that a high amine content in crude ergot results from deterioration and decomposition of the specific alkaloids of the drug during storage. It has been conclusively shown in these studies that the amine activity is entirely independent of the alkaloidal content. Samples unusually high in alkaloids are frequently correspondingly high in the non-specific amines, although the results in Tables I and II clearly show that the amine activity is entirely independent of the alkaloidal content. Furthermore, aqueous preparations permitted to undergo fermentation or putrefaction (Table II) show a pronounced increase in amine content and at the same time a slight increase in alkaloidal activity. The increase in the alkaloidal content is undoubtedly due to acid formation during the fermentation which increases the efficiency of the water as an extraction menstruum. The increased amine activity is due to fermentation or putrefaction of substances in the ergot other than the active alkaloids. The amine and alkaloidal changes which take place in crude ergot under varying conditions of storage will be considered in a succeeding article.

RESULTS OBTAINED.

Typical results obtained during these studies are incorporated in Tables I and II. To tabulate the data on all samples studied would unduly extend the length of this report. The ten samples of crude ergot included in Table I were obtained from importations. It will be noted that these samples are not intended especially to typify the general run of importations of the various varieties of ergot regarding activity, but merely to show the amine and alkaloidal activity of the crude drugs entering into the preparation and study of the Aqueous Extracts embraced in Table II.

The "Crude Drug No." in Table I corresponds to the number under the same heading in Table II.

TABLE II.—PHARMACO-DYNAMIC ACTIVITY OF AQUEOUS EXTRACTS PREPARED FROM CRUDE ERGOTS OF TABLE I.*

Crude drug no.	Aqueous preparation.	Potency by Cock's Comb Method (24, 15). U. S. P. Potency for Fluidextract.** Old cockerels. *Per cent.*	New cockerels. *Per cent.*	Total Alkaloids, Calculated as Ergotamine** Base. Broom-Clark Method (28). *Per cent.*	Isolated Guinea-Pig Uterus Method for alkaloids (39). *Per cent.*	Total Amines, Calculated as Histamine.** Isolated Guinea-Pig Uterus Method for amines (38). *Per cent.*
I	(*1*) Extractum Ergotæ (Pilular) B. P. Method:					
	(*a*) Allowed to ferment	33	10	0.005	††	0.045
	(*b*) Not allowed to ferment	20†	20†	0.003	††	0.025
	(*2*) Extractum Ergotæ Liquidum, B. P. Method:					
	(*a*) Allowed to ferment	20†	25	0.008	††	0.050
	(*b*) Not allowed to ferment	20	20	0.006	††	0.030
	(*3*) Extractum Ergotæ Aquosum, N. F.:					
	(*a*) N. F. Method—evaporated to pilular consistence	20†	20†	0.005	††	0.025
	(*b*) N. F. Method—not evaporated	20†	20	006	††	0.030
	(*c*) Same as (*b*), except drug de-fatted with petroleum benzine before extraction	20†	20	0.006	††	0.035
	(*4*) Alkaline Aqueous Extract of Ergot (3% sodium bicarbonate menstruum—de-fatted before extraction, 20% alcohol added to finished product):					
	Not allowed to ferment	20	20†	0.002†	††	0.035
	(*5*) Acid Aqueous Extract of Ergot (same as No. 4 except 0.2% hydrochloric acid U. S. P. in water used as menstruum, 20% alcohol added as in No. 4):					
	Not allowed to ferment	50	70–80	0.025	0.02	0.030
II	(*1*):					
	(*a*)	20	20†	0.004	††	0.030
	(*b*)	20†	20†	0.003†	††	0.009
	(*2*):					
	(*a*)	20†	20	0.006	††	0.040
	(*b*)	20†	20†	0.004	††	0.015
	(*3*):					
	(*a*)	20†	20†	0.003	††	0.008
	(*b*)	20†	20	0.006	††	0.010
	(*c*)	20	20	0.008	††	0.015
	(*4*):	20†	20†	0.002†	††	0.015
	(*5*):	100–120	100–120	0.042	0.04	0.013

TABLE II.—(*Continued*).

Crude drug no.	Aqueous preparation.	Potency by Cock's Comb Method (24, 15). U. S. P. Potency for Fluidextract.** Old cockerels. *Per cent.*	New cockerels. *Per cent.*	Total Alkaloids, Calculated as Ergotamine** Base. Broom-Clark Method (28). *Per cent.*	Isolated Guinea-Pig Uterus Method for alkaloids (39). *Per cent.*	Total Amines, Calculated as Histamine.** Isolated Guinea-Pig Uterus Method for amines (38). *Per cent.*
III (*1*):						
(*a*)		30–40	20†	0.005	††	0.063
(*b*)		20	10	0.003	††	0.037
(*2*):						
(*a*)		40–50	25	0.007	††	0.072
(*b*)		20†	20†	0.003	††	0.045
(*3*):						
(*a*)		20	20†	0.004	††	0.037
(*b*)		10†	20†	0.005	††	0.040
(*c*)		20†	20†	0.007	††	0.045
(*4*):		20–30	20†	0.002†	††	0.045
(*5*):		40–50	70–80	0.027	0.02	0.042
IV (*1*):						
(*a*)		30–40	10–20	0.006	††	0.050
(*b*)		10–20	20†	0.004	††	0.027
(*2*):						
(*a*)		20–30	20†	0.008	††	0.068
(*b*)		20†	20†	0.005	††	0.032
(*3*):						
(*a*)		20†	20†	0.003	††	0.027
(*b*)		20	20†	0.004	††	0.035
(*c*)		20†	20†	0.005	††	0.033
(*4*):		20	20†	0.002†	††	0.033
(*5*):		100	120	0.047	0.04	0.030
V (*1*):						
(*a*)		30–50	20	0.006	††	0.087
(*b*)		20†	20†	0.003	††	0.042
(*2*):						
(*a*)		20†	25	0.010	††	0.100
(*b*)		20†	20†	0.005	††	0.052
(*3*):						
(*a*)		20	20†	0.003	††	0.037
(*b*)		20†	20†	0.006	††	0.050
(*c*)		20†	20†	0.006	††	0.050
(*4*):		25	20†	0.002†	††	0.052
(*5*):		100	160–170	0.066	0.06	0.050
VI (*1*):						
(*a*)		20†	20†	0.007	††	0.052
(*b*)		20†	20†	0.003	††	0.022
(*2*):						
(*a*)		20†	25	0.010	††	0.065
(*b*)		20†	20†	0.006	††	0.025
(*3*):						
(*a*)		20†	20†	0.002	††	0.020
(*b*)		20†	20†	0.004	††	0.026
(*c*)		20†	20†	0.004	††	0.027
(*4*):		20†	20†	0.002†	††	0.027
(*5*):		200	200	0.062	0.06	0.027

TABLE II.—(*Concluded*).

Crude drug no.	Aqueous preparation.	Potency by Cock's Comb Method (24, 15). U. S. P. Potency for Fluidextract.** Old cockerels. *Per cent.*	New cockerels. *Per cent.*	Total Alkaloids Calculated as Ergotamine** Base. Broom-Clark Method (28). *Per cent.*	Isolated Guinea-Pig Uterus Method for alkaloids (39). *Per cent.*	Total Amines, Calculated as Histamine.** Isolated Guinea-Pig Uterus Method for amines (38). *Per cent.*
VII (*1*)						
(*a*)		20†	20†	0.008	††	0.047
(*b*)		20†	20†	0.002	††	0.009
(*2*):						
(*a*)		20†	20	0.015	††	0.075
(*b*)		20†	20†	0.004	††	0.010
(*3*):						
(*a*)		20†	20†	0.003	††	0.007
(*b*)		20†	20†	0.004	††	0.011
(*c*)		20	20	0.006	††	0.012
(*4*):		20†	20†	0.002†	††	0.012
(*5*):		100	120	0.040	0.04	0.012
VIII (*1*):						
(*a*)		25	40	0.012	††	0.155
(*b*)		25	20	0.005	††	0.072
(*2*):						
(*a*)		25	50	0.015	††	0.167
(*b*)		30–40	20†	0.006	††	0.079
(*3*):						
(*a*)		20	20†	0.005	††	0.068
(*b*)		30–40	25	0.006	††	0.082
(*c*)		25	30–40	0.008	††	0.084
(*4*):		50	20†	0.002†	††	0.087
(*5*):		100–150	175–200	0.067	0.06	0.085
IX (*1*):						
(*a*)		60–80	30–40	0.006	††	0.175
(*b*)		30–50	20	0.004	††	0.115
(*2*):						
(*a*)		60–80	20–40	0.008	††	0.189
(*b*)		30–40	30–50	0.006	††	0.125
(*3*):						
(*a*)		30–40	20	0.005	††	0.118
(*b*)		30–50	30–50	0.005	††	0.123
(*c*)		20	25	0.006	††	0.123
(*4*):		60–70	30–40	0.002†	††	0.125
(*5*):		100	160–175	0.067	0.06	0.125
X (*1*):						
(*a*)		60–80	50	0.006	††	0.175
(*b*)		50–60	20–40	0.003	††	0.133
(*2*):						
(*a*)		70–80	50–70	0.008	††	0.183
(*b*)		50–60	30–40	0.005	††	0.147
(*3*):						
(*a*)		50–60	30–40	0.002	††	0.130
(*b*)		50–60	50	0.004	††	0.127
(*c*)		20–60	50–60	0.005	††	0.150
(*4*):		50–75	20–30	0.002†	††	0.150
(*5*):		80–150	200	0.083	0.08	0.150

* Concentration of all preparations adjusted so that 1 cc. represents 1 gram of crude Ergot. Pilular extracts were redissolved in water in a concentration such that 1 cc. of solution represents 1 gram crude Ergot. Expressions of activity or constituent content therefore represent the amounts that were derived from the crude drug by the methods of preparation described.

** Standards Involved in Tables I and II:

(*a*) For total alkaloids—Ergotamine Tartrate (Sandoz Chemical Co.) corresponding to 84.5% Ergotamine base.

(*b*) For total amines—Histamine (Pfanstiehl Chemical Co.)

(*c*) Cock's Comb Potency. Compared with the U. S. P. X Standard Fluidextract of Ergot prepared and distributed to pharmaceutical manufacturers and scientific investigators by this laboratory, equivalent to 0.03 per cent Ergotamine base.

† Less than.

†† No results.

The geographical origin or variety of the Ergot of Rye is given in the second column under "Commercial Source." (Table I.)

The U. S. P. X Cock's Comb potency (Table I) was determined by the U. S. P. X method of preparing a fluidextract in the prescribed manner and determining the potency by the Cock's Comb Method. "Old cockerels" are birds that had been standardized and used for ergot testing every two weeks for six months or more, but still within U. S. P. specifications for weight. "New cockerels" are new birds that had not been used except for the few injections at 2-week intervals necessary for standardization. Expressions of potency are based on a comparison of the fluidextract yielded by the crude drug sample with the U. S. P. Standard Fluidextract of Ergot prepared by the Pharmacological Laboratory. The Cock's Comb potency of the aqueous extracts (Table II) is also expressed in terms of the Standard Fluidextract. The value of the Standard Fluidextract in terms of ergotamine will be shown in a later report. Total alkaloids of crude drug, calculated as ergotamine base, by the Broom-Clark Method, modified (28) and by the Isolated Guinea-Pig Uterus Method for Alkaloids (39) were determined on the fluidextract of the crude drug (Table I) and on the aqueous extracts (Table II). Total amines, calculated as histamine (Table I) were determined by the Isolated Guinea-Pig Uterus Method for Amines of Crude Ergot (38). The amine content of the aqueous extracts in Table II were obtained by the direct application of the method for aqueous extracts (38).

The results in Tables I and II are obtained from concentrations such that 1 cc. of preparation represents 1 gram of crude ergot. The semi-solid or pilular extracts were adjusted to the same concentration, that is, the pilular extract obtained from 100 grams of crude ergot was redissolved in enough water to make 100 cc. of preparation.

A brief explanation of the fermented and non-fermented aqueous preparation (Table II) is given earlier in this article. If chloroform water is used as the menstruum, as in the N. F. method of preparation, no fermentation takes place during the process of extraction. When pure water is used, and extraction is carried out at ordinary room temperature, fermentation and putrefaction rapidly take place. If the extraction is carried on at a low temperature (5° C. to 10° C.), the fermentation and putrefaction are prevented. Several preparations of each type are found in Table II. The finished liquid extracts undergo further fermentation or putrefaction unless at least 15 per cent of alcohol is added or the preparation is stored in a

refrigerator. This procedure was observed in these studies, as it was found that the further the enzyme action progressed the greater the amine content became. Even in the preparations "allowed to ferment," the fermentation was permitted only during the extraction process.

It was found to be almost impossible to exhaust the drug of amine activity as long as the fermentation was allowed to continue, because in some cases active amines were formed nearly as rapidly as they were removed by the slow percolation (15 to 20 drops per minute). If the fermentation is prevented, there is little difficulty in exhausting the drug of amines. Exhaustion of the drug was tested by the Isolated Guinea-Pig Uterus Method for Amines of Ergot (38).

CAROTID BLOOD PRESSURE STUDIES OF AQUEOUS EXTRACTS OF ERGOT ON ANESTHETIZED DOGS.

The effects of the various constituents of ergot upon the blood pressure of experimental animals are given in several of the available texts on pharmacology (26, 14). Briefly, the pressor reactions of the most important active constituents on anesthetized dogs, by intravenous administration, are as follows:

(1) Alkaloids:

(*a*) Ergotamine produces a rather persistent rise in blood pressure in doses of 0.02 to 0.1 milligram per kilogram of dog (Plate VIII). Diminishing response results from repeated dosage.

(*b*) Ergotoxine has the same reactions as ergotamine.

(2) Amines:

(*a*) Histamine produces a sudden but rather transitory fall in blood pressure in doses as low as 0.01 milligram per kilogram of dog. Larger doses cause proportionately greater effects in persistence and magnitude. A dose of 0.05 milligram per kilogram of dog produces a very sudden and pronounced fall in blood pressure (Plate IX).

(*b*) Tyramine produces a somewhat transitory rise in blood pressure, similar to, but a bit more persistent than that caused by epinephrine. It is less than one-tenth as active on blood pressure as histamine or epinephrine. The rise in blood pressure from tyramine is not nearly as persistent as from the ergot alkaloids.

All of the aqueous extracts listed in Table II produced an effect upon the blood pressure of anesthetized dogs similar to that shown in Plate X, which gives the results of a fermented extract. The various aqueous extracts differed only in potency. The potency observed by this method was closely parallel to that determined by the Isolated Guinea-Pig Uterus Method for Amines (Table II). The resultant activity apparently was due almost entirely to the amines, the most predominant of which was histamine, as a sudden fall in blood pressure similar to that produced by histamine was invariably produced, masking any rise in blood pressure that could result from the small amounts of alkaloids or tyramine that may have been present.

Plate XI shows how histamine masks the effect of ergot alkaloids, the two substances being antagonistic in their apparent individual effects upon blood pressure.

The amine potency by the blood pressure method was found to increase the more the preparation was permitted to ferment, as was also observed by the Guinea-Pig Uterus Method for Amines (Table II).

Plate 8.—Showing the persistent rise in blood pressure produced by the intravenous administration of 0.25 mg. of Ergotamine Tartrate. Dog weighed 10.7 Kg.

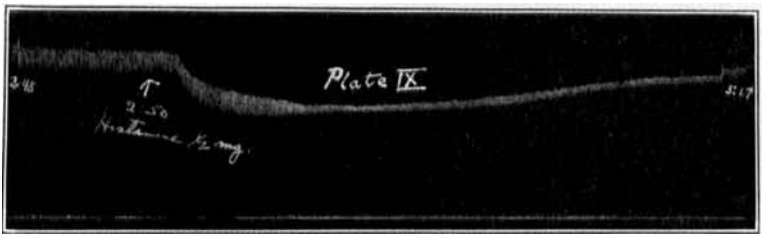

Plate 9.—The sudden and pronounced fall in blood pressure produced by the intravenous administration of 0.5 mg. of Histamine to dog weighing 11.2 Kg.

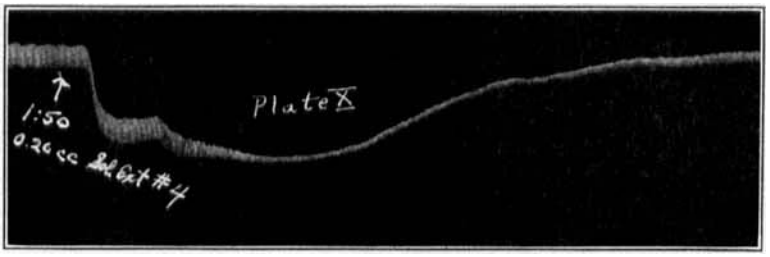

Plate 10.—The effect produced by the intravenous administration of 0.2 cc. of solution of an Aqueous Extract of Ergot (fermented) (1.0 cc. representing 1.0 Gm. Ergot). Note the similarity of this fall in blood pressure and that shown in Plate IX. Dog weighed 9.7 Kg.

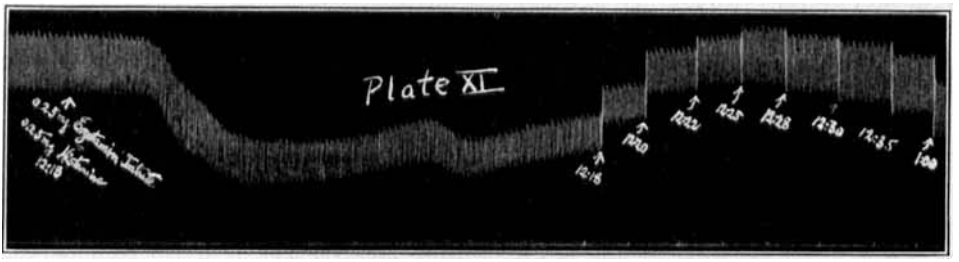

Plate 11.—The effect produced by a dose of 0.25 mg. each of Histamine and Ergotamine Tartrate in mixture injected intravenously. Dog weighed 9.7 Kg.

DISCUSSION OF RESULTS.

Tables I and II and plates are practically self-explanatory. It is clearly evident that methods of preparation involving the use of water or alkalinized water as the extraction menstruum (Table II) yield extracts which may be high in amine activity but which are practically devoid of specific ergot alkaloids.

The amine content of crude ergot varies enormously. Some samples were practically devoid of amines but most of them contained very appreciable amounts. This amine activity apparently is entirely independent of the alkaloidal content in crude ergot. The aqueous preparations not permitted to ferment usually contained all of the amines (histamine, tyramine, etc.) present in the parent drug, but only insignificant amounts of the alkaloids. The aqueous preparations permitted to ferment had a much higher amine activity than the parent drug. The longer the aqueous preparations ferment the higher the amine content becomes. This fermentation or putrefaction is evidenced by the evolution of gas and the development of putrid odors. It results in the formation of acid, which slightly increases the efficiency of water as a solvent for the specific ergot alkaloids.

The direct addition of acid to the aqueous menstruum increases the efficiency of water in extracting the alkaloids. The amines are also completely soluble in acid menstruum. Mildly alkaline aqueous menstrua extract the amines, but exclude the alkaloids from the resulting preparations.

The Isolated Guinea-Pig Uterus Method for detecting and estimating the non-specific amine activity of ergot preparations (38) gives results closely paralleling those obtained by pressor studies and is reasonably accurate in estimating the amine activity of aqueous extracts.

Blood pressure studies indicate that histamine is the most important of the constituents found in aqueous extracts of Ergot, so far as activity and occurrence are concerned, although it is highly probable that tyramine, cholines, etc., may also be present. The greater activity of histamine by uterine and pressor methods makes detection of smaller amounts of other principles impossible by these methods. Histamine is synergistic with tyramine and acetyl choline in its action on the isolated guinea-pig uterus, but is antagonistic to tyramine and the alkaloids on the carotid blood pressure of anesthetized dogs.

The Isolated Guinea-Pig Uterus Method for Ergot Alkaloids was not sufficiently sensitive to measure the small amount of alkaloids found in these aqueous preparations of relatively high amine activity. The Acid Aqueous Extracts (No. 5 in Table II) alone contained a measurable quantity of alkaloids.

The results obtained by the Cock's Comb Method upon the aqueous extracts are interesting and decidedly contrary to those expected, as this method is generally held to be capable of measuring only the alkaloidal activity of ergot preparations. The values in Table II indicate that aqueous preparations may show appreciable activity by this method, even though practically no alkaloids are present. The aqueous preparations with the exception of the "Acid Aqueous Extracts," showing greatest activity on the Cock's Comb are high in amine activity only, suggesting that certain of the amines are present in sufficient amounts to cause the bluing of the comb. The old cockerels reacted to the amines to a greater degree than the new cockerels, although the results obtained were not consistent. The acid or fermented

aqueous extracts which contained higher proportions of akaloids frequently gave lower results than those containing practically no alkaloids. The results in Table II conclusively show that the Cock's Comb Method is not reliable for testing *aqueous extracts*. A more thorough study of the effects of the various constituents, individually and in mixtures, upon White Leghorn cockerels will receive consideration in the next article of this series. The discordant results obtained by this method in the assay of the crude drugs (as the fluidextract) upon the old cockerels and new cockerels will also be considered more thoroughly. Table I was included in this article merely to show that the crude drug samples involved were active.

The Broom-Clark Isolated Rabbit Uterus Method, using the washing technique described by Pattee and Nelson (28) yielded consistent results in measuring even the small amounts of ergot alkaloids in aqueous extracts of ergot. The washing of the uterine strips before observing the final epinephrine response can be made to effectually obviate amine interference, although several washings were occasionally necessary.

The amines of ergot apparently are completely extracted by either mildly alkaline or acid aqueous menstrua, but the alkaloids were not extracted in significant amounts by water, chloroform water, or mildly alkalinized water. Mildly acidified water or strong solution of the alkali hydroxides extracts higher proportions of the alkaloids. Concentrating the aqueous extracts and precipitating with alcohol detracts but little from the amine or alkaloidal activity of the extracts, as both amines and alkaloids are soluble and not precipitated or removed by the treatment with alcohol and the subsequent filtration. Further concentration at a moderate temperature to a semi-solid or pilular consistence results in a loss in both amine and alkaloidal potency, although the loss is not great, usually less than 30 per cent, although even this deterioration practically prevents the appearance of active alkaloids in the soft extract.

Permitting the drug to ferment during extraction with the aqueous menstrua results in marked increase in the amine activity, and a slight increase in aklaloidal content owing to acid formation which increases the solubility of the alkaloids. The use of chloroform water, U. S. P., prevents this fermentation, as does the preparation of the extract in a cold room. The natural acidity of the drug provides for a very slight extraction of ergot alkaloids by water if this acidity is not neutralized by the addition of alkalies.

If, as is quite generally believed, the alkaloids are the principles of ergot which are responsible for the desirable clinical activity of its preparations, the processes specified by the N. F. and B. P. for the preparation of Extractum Ergotæ Aquosum and Extract of Ergot or Liquid Extract of Ergot appear to be decidedly faulty with respect to the menstruum employed.

Furthermore, if the amines, such as histamine and tyramine, are the desirable constituents of ergot, which is highly improbable, as they are practically inactive on the uterus when administered orally (14), there would be no reason for retaining ergot in the Pharmacopœia, since these substances can be obtained much more cheaply and in a purer state from other sources. This supposition, however, has been quite definitely disproven by the numerous investigations of recent years. There is abundant evidence, published and unpublished, definitely establishing the clinical value of ergot and the fact that this value is due to the specific alkaloids

contained therein. Since this investigation has shown that B. P. and N. F. Aqueous Extracts of Ergot prepared by the processes specified do not contain significant amounts of the ergot alkaloids, it is believed that these preparations are practically without value with respect to ergot therapy. The appearance of these aqueous extracts on the market has provided for their use by clinicians and physicians, usually with little or no success, resulting in a subsequent undue condemnation of all types of ergot preparations and a search for a suitable substitute.

(To be continued)

HAZINESS OF FINAL CHLOROFORMIC EXTRACTIONS IN ALKALOIDAL ASSAYING.*

BY GEORGE E. ÉWE.

In estimating alkaloids, as a rule, the alkaloids are eventually segregated in an ammoniacal, aqueous liquid containing large quantities of ammonium sulphate. The alkaloids are then "shaken out" with an immiscible solvent, chloroform usually being the choice. These chloroformic extractions, instead of being brightly clear, are often hazy. This haziness, when not due to the presence of solid matter, is due to an almost colloidal suspension of a trace of the aqueous liquid from which the alkaloids were extracted. Since this aqueous liquid contains ammonium sulphate, and since, under the influence of heat and moisture, alkaloids tend to liberate ammonia from ammonium sulphate (and are thereby proportionately neutralized by the sulphate radicle), it seems important to ascertain the possible effect of this latter type of haziness of final chloroformic extractions upon the results of alkaloidal assays.

The following experiments illustrate the effect of alkaloid upon ammonium sulphate under several varied conditions: When a grain or two each of strychnine alkaloid and ammonium sulphate are placed in a 100-cc. volumetric flask along with about 15 cc. of water, or water-saturated chloroform, and a piece of moistened red litmus paper is placed between pledgets of cotton in the neck of the flask and the mixture then boiled, the litmus paper turns blue, due to liberation of ammonia from the ammonium sulphate by the alkaloid. When the alkaloid and ammonium sulphate are dried by heat and the chloroform is essentially deprived of moisture by boiling down to half its volume, and the above test is repeated, a very doubtful alkaline reaction is obtained, if any. No reaction is obtained in this test from mixtures of ammonium sulphate and water-saturated chloroform, or strychnine alkaloid and water-saturated chloroform, or dried ammonium sulphate and dried chloroform or dried alkaloid and dried chloroform.

To ascertain if haziness of the final chloroformic extracts has any influence upon results of alkaloidal assays, the final chloroformic extracts, from alkaloidal assays which happened to be hazy, were brought to a definite volume and divided into three equal aliquots. One aliquot was evaporated to 5 cc. excess tenth-normal sulphuric acid added, the chloroform boiled off completely and the excess of acid titrated back with fiftieth-normal sodium hydroxide, using methyl red as indicator. The second aliquot was either washed with 10 cc. of water in a separator

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