# THE BIOLOGICAL ASSAY OF ERGOT PREPARATIONS

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## I. INTRODUCTION

The present United States Pharmacopoeia X, hereafter called the U. S. P., requires that the official Fluidextract of Ergot be assayed by the cock's comb method. Methods are not prescribed for the other ergot preparations since no others are official. It was shown some time ago (1) that of the then known constituents of ergot only ergotoxin would produce the characteristic cyanosis and gangrene of the cock's comb. Since the recent isolation of a second active alkaloid, ergotamine, it has been shown that this substance also will produce the characteristic discoloration and in sufficient doses gangrene of the rooster's comb and wattles (2) The U. S. P. method then is an index of the concentration of the two alkaloids, ergotoxin and ergotamine, peculiar to ergot.

More recently there have been introduced a number of assay methods based on the property of these same alkaloids to paralyze or to reverse the response of various tissues to epinephrine (3, 4). Of these methods, the one which has been most extensively used is that of Broom and Clark (4), in which the strength of an ergot preparation is determined by its ability to decrease the response of the isolated uterus of the rabbit to constant doses of epinephrine. Although this method has been examined by a number of investigators, except for a short series in the original paper of Broom and Clark (4), no comparison of the results of assays by the two methods seems to have been made. The present study was undertaken to supply that lack. As it has

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progressed a number of observations have been made which seem to the writers worthy of record. Some modifications of the methods have been found necessary, and are given in detail. Finally, the value of the Standard Fluidextract of Ergot of the U. S. P. has been evaluated in terms of ergotamine, and ergotamine and ergotoxin have been compared with each other. These various points will be considered in separate sections.

## II. THE COCK'S COMB METHOD

The U. S. P. outlines only in a general way the method of carrying out an assay by this method. According to it, a satisfactory ergot preparation is one which gives the same degree of discoloration in the combs of white Leghorn cocks of specified age and weight as is produced by 0.5 cc. per kilogram of a standard fluidextract, injected intramuscularly. This statement, while not an exact quotation, does include the details given by the U. S. P. As pointed out by Gittinger and Munch (5), these directions need to be supplemented. In the first place, not all birds give a satisfactory degree of discoloration with this dose of the standard fluidextract. At the time of writing their monograph on the subject of ergot assay, Edmunds and Hale (6) stated that there was little evidence in the literature bearing on this point of variation in reaction of different roosters of the same breed. In the series of 30 birds used in our study, 12 required more than 0.5 cc. per kilogram to produce what we considered an acceptable color change. In the larger series of 190 birds reported by Gittinger and Munch, 28 per cent required doses of 0.6 cc. or more. It therefore becomes necessary to determine by the injection at proper intervals of varying amounts of a standard ergot preparation just what dose will produce an acceptable degree of bluing. When this has been done for each of the roosters, they are ready for use in assaying unknown preparations. This dose, however, cannot be considered as permanently determined, for the ergot threshold changes, either from age or repeated injection. Our data do not justify any conclusions as to which factor is responsible, but we noted that the effective dose increased as much as 100 per cent for some birds in

the eight months our pen of cocks was used. It is obvious that this variation may be a cause of error in determinations.

Finally, the sensitivity of each cock to variations in dosage is not known and cannot be determined readily, since one must carry his idea of the degree of color change in a particular cock from one injection period to the next. (We have used the code described by Gittinger and Munch for recording color changes.) Edmunds and Hale felt that by proper care one could detect variations of 10 per cent in the dosage by this method. The experiment reported below illustrates the difficulty we have had in making such a discrimination.

*April 16, 1928.* A series of 9 cocks, whose reaction to U. S. P. Standard Fluidextract No. 635 from the Bureau of Chemistry, had previously been determined, was injected with ergotamine methanesulphonate as indicated in the table. At sixty and again at ninety minutes the cocks were arranged according to intensity of discoloration. The results are given as follows:

| COCK NUMBER | ERGOT THRESHOLD | DOSE | DOSE CORRECTED FOR ERGOT THRESHOLD | ORDER AT 60 MINUTES | ORDER AT 90 MINUTES |
|---|---|---|---|---|---|
| | *cc. per kgm.* | *mgm. per kgm.* | | | |
| 19 | 0.95 | 0.10 | 0.19 | 3 | 3 |
| 15 | 0.8 | 0.15 | 0.24 | 2 | 1 |
| 16 | 0.8 | 0.20 | 0.32 | 1 | 2 |
| 11 | 0.7 | 0.25 | 0.35 | 7 | 6 |
| 1 | 0.7 | 0.3 | 0.42 | 4 | 4 |
| 14 | 0.6 | 0.35 | 0.42 | 6 | 7 |
| 4 | 0.6 | 0.4 | 0.48 | 5 | 5 |
| 17 | 0.6 | 0.45 | 0.54 | 8 | 8 |
| 6 | 0.5 | 0.5 | 0.50 | 9 | 9 |

The reaction in cocks 19, 15 and 16 was apparent, but not of a degree equivalent to that taken as standard discoloration. Since 0.25 mgm. in no. 11 was the smallest dose to give this change, this dose was taken as corresponding to the U. S. P. Standard Fluidextract. Actually, however, the discoloration produced by this dose was greater than that produced by doses of 0.3 and 0.4 mgm. at the end of ninety minutes. These doses are not only larger as calculated, but as corrected for the difference in ergot threshold. The reactions of roosters nos. 11, 1, 14

and 4 were all of the degree taken as standard although no. 4 received a 60 per cent larger dose than no. 11.

If the correct dose be taken as 0.25 mgm. per kilogram then the official U. S. P. Standard Fluidextract contains 0.5 mgm. of alkaloids per cubic centimeter. This value will be referred to later.

Using the method as above described, we have made some 275 injections into 30 different cocks and have determined values for some 18 preparations. The results obtained are tabulated together with those obtained by the Broom-Clark method and will be discussed under a separate heading.

### III. THE BROOM-CLARK METHOD

As this method of assay is carried out, one uses a series of pairs of similar strips of rabbit uterus, suspended a pair at a time in separate chambers in oxygenated Ringer's solution at 38°C. and arranged to record in the usual manner. The epinephrine response of both strips is determined, and then to one is added a definite amount of ergot preparation serving as a standard, and to the other a measured amount of the preparation being assayed. After the proper lapse of time the response to epinephrine is again determined for both strips. The lesser response indicates the greater paralysis. By varying the relation of the two ergot doses and keeping all other factors constant, in a series of comparisons amounts of unknown and standard ergot are found which will produce the same degree of paralysis. The strength of the two preparations are then in inverse proportion to the doses required.

Details of the method are given in the original report (4) and in the other papers cited, and will be repeated here only when our practice has been at variance with or our results have suggested modifications of the technique of others.

The present report is based on the use of some 280 strips from 80 rabbits, including those that proved to be unsatisfactory.

#### *Apparatus*

We have used two glass chambers of 100 cc. capacity each for suspension of the isolated strips of uteri. These are mounted in

a constant temperature bath in the usual way, and arranged to fill and empty from below. The only alteration we have made is to arrange reservoirs in the bath of a size sufficient to warm enough Ringer's solution to wash out the suspension chambers three or four times. Using 100 cc. chambers requires a relatively large amount of Ringer's solution, and smaller chambers no doubt would be just as satisfactory on other scores and would avoid this prodigal use of Ringer's solution.

*Muscle*

Our experience confirms that of Burn at this point. The uteri of large rabbits 3 to 4 kgm. in weight, taken several weeks post-partum, are the most satisfactory for use. Uteri of smaller rabbits, either nulliparous or multiparous, may be used, but do not work as well. Pregnant uteri and those obtained shortly after parturition are usually too active to be of value. Occasionally we have found in old multiparous rabbits thin-walled atonic uteri, which have so uniformly proved unsatisfactory that we no longer attempt to use them. The uteri can be kept on ice for as long as six days, though it is preferable to use them before such a period has elapsed. From large uteri eight to ten or more pairs of strips may be obtained.

Since much valuable time is wasted if unsatisfactory uteri are used, it is important to be able to determine by external examination those animals whose uteri are likely to prove satisfactory. Since each pregnancy leaves some subinvolution of the uterus and at the same time an eversion and thickening of the vaginal walls, one may often ascertain the approximate condition of the uterus by the appearance of the vaginal orifice. If the walls are thickened and somewhat everted, the uterus is usually of sufficient size to give good results. If a distinct hyperemia is present, the uterus will also be found to be congested and probably too active to be altogether satisfactory. Lactating animals are not satisfactory. Pregnancy can be detected in the later stages by palpation. It is to be suspected if the vaginal walls are swollen and have a purplish hue.

*Method of performing assay*

The size of the uterine segment used depends on the muscle development. Ordinarily it is divided into two unequal parts by making two parallel cuts on either side of the mesometrium. (This is spoken of as the mesentery by several of the writers who have used this method.) The smaller part, that to which the mesometrium attaches, is discarded, as it does not relax satisfactorily. The remaining piece is again divided by a longitudinal cut into two equal parts, which are then mounted and arranged to record contractions of the longitudinal muscle in the usual manner.

The tension used is of importance. It must be sufficient to produce relaxation but not enough to prevent a good contraction when epinephrine is added. Only by experience can one determine how to regulate it. When properly adjusted the spontaneous contractions are small, but addition of a proper dose of epinephrine produces a prompt increase in tone which is maintained for a time and then gradually returns to the base line.

It is stated that the response of the rabbit's uterus to epinephrine is constant. In our experience, not infrequently the amplitude of contraction to constant doses of epinephrine increases for several injections before it becomes constant. Broom and Clark's figure 2, although given to show the constancy of response to epinephrine, actually shows such an increase through the first four doses. On the other hand, occasionally it is found that the response falls off rapidly. We have been convinced, therefore, that it is necessary to verify the constancy of response by trial before reducing it with ergot or its alkaloids. Having determined by trial the dose necessary and the constancy of response to this dose, one is then ready to add ergot or ergot alkaloids to the two chambers. It is convenient to add the standard, whether a fluidextract (diluted) or a solution of one of the specific alkaloids, always to the same bath, and the unknown to the other. The time, also, is carefully noted, and it has been our practice to keep the changes in one bath just exactly one minute later than in the other. After the ergot or alkaloid has acted exactly five minutes, it is washed, and after another five minutes the

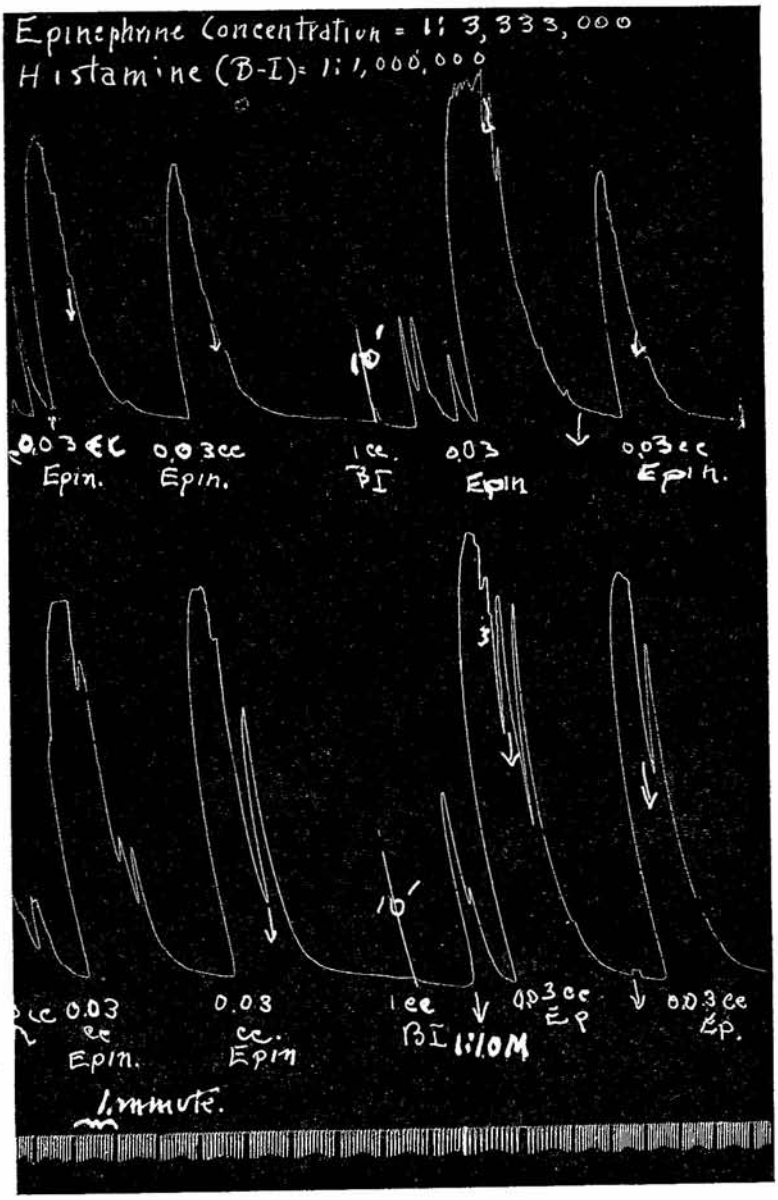


FIG. 1. TWO STRIPS FROM THE SAME UTERUS

The upper strip is treated with epinephrine twice, and then a non-stimulant dose of histamine allowed to act for ten minutes, the length of time that ergot is allowed to act in an assay. Epinephrine is again added, without washing off the histamine. The response is much increased. After washing once the epinephrine response returns to the original. On the lower strip the procedure is the same except that the histamine is washed off at the end of the ten minutes. Control experiments showed that the ten-minute rest did not of itself bring about such a change in response.

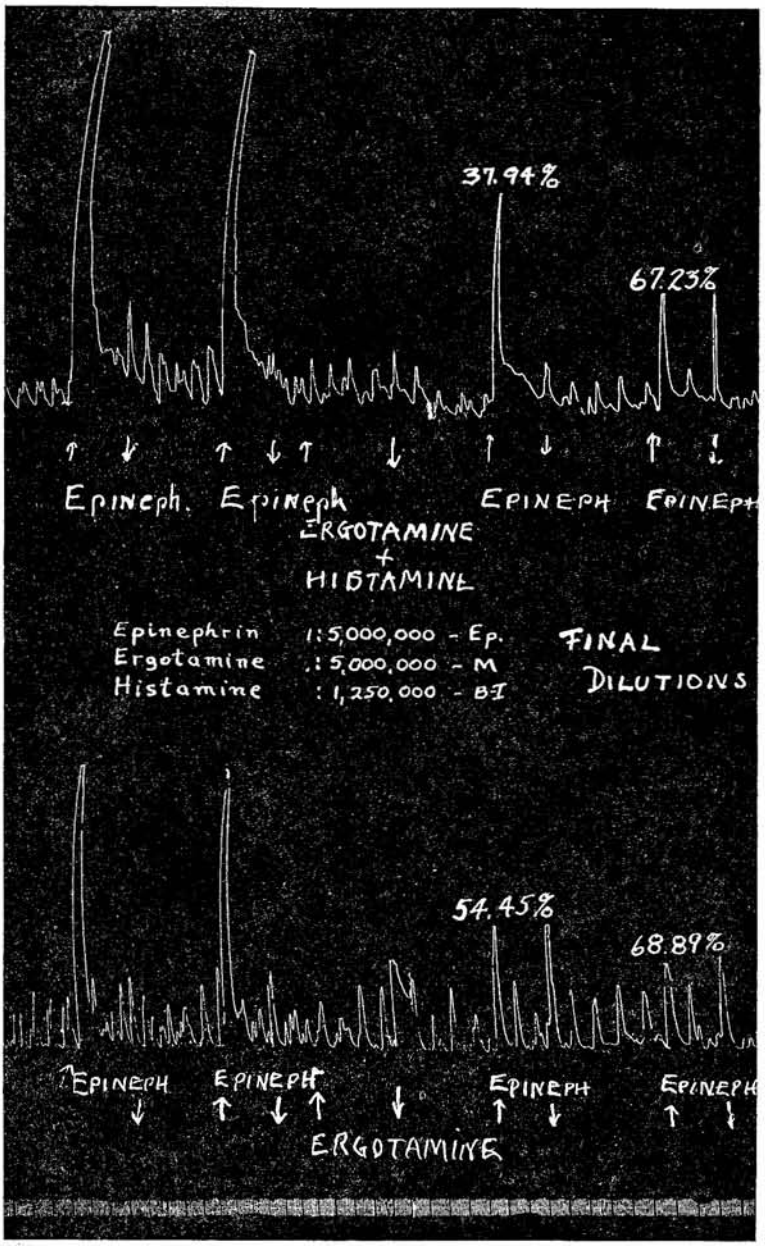


FIG. 2. EXPERIMENT IN WHICH THE SAME CONCENTRATION OF ERGOTAMINE WAS ADDED TO BOTH STRIPS, BUT HISTAMINE ONLY TO THE UPPER ONE

The first contraction of the histamine treated strip is too large but the second agrees to an unusual extent with the second contraction of the strip not treated with histamine.

previously determined dose of epinephrine tried again. When its effect has been obtained, it is washed off and at the end of another five minutes repeated as a check. The second contractions are usually less than the first following ergot.[2]

The dose of ergot used should be sufficient to produce a reduction of from 25 to 50 per cent in the epinephrine response. If the reduction is greater than 50 per cent in a strip showing any marked degree of spontaneous contraction, it may be difficult to determine the percentage of reduction. If, however, the dose of ergot used should happen to produce a complete paralysis, one may in some cases get some index of the relative strength of the two by adding successively larger doses of epinephrine to both strips until one of them responds. Obviously, the one responding first was treated with the lesser amount of ergot alkaloids. We have found that an average fluidextract in a dilution of 1:2,500 will usually produce a sufficient degree of reduction of the contraction produced by epinephrine in a 1:2,500,000 dilution. This dilution of epinephrine will prove insufficient for some strips and too much for others. Langecker (8) has studied this in detail, and in 172 strips found the minimum effective dose to be more than 1:2,000,000 in about 6 per cent. If it is necessary to vary the epinephrine from the usual value, it will be necessary to change the ergot dilution accordingly. According to Gaddum (9) and Mendez (10) there is a direct relation between the concentration of epinephrine and the concentration of ergotamine required to reduce it a specific amount. We have not paid particular attention to this point, but in some cases it has seemed that the greater the sensitivity of a strip to epinephrine, the *greater* the concentration of ergot alkaloids necessary to affect the epinephrine response. This, if correct, would not agree with the above mentioned authors.

We have varied the procedure of others (Burn and Ellis) in that instead of adjusting the tension on the two strips until they give similar responses, we measure the heights of the epinephrine curves on the kymograph record before and after the ergot is added with dividers, and when the *percentage* reductions are equal,

[2] Mendez (10) recommends a ten-minute period for the action of ergot.

the ergot strengths in the two chambers are taken as equal. This variation adds no new factor, and saves some adjustments. Of course, it often happens that the two strips give essentially equal contractions, since the attempt is always made to have them as nearly alike as possible.

*Difference between ergot and ergot alkaloids*

In checking the accuracy of the method of assay as described by others, we found that when ergotamine was used in both chambers, it was possible to come within the 10 per cent of the correct value. On repeating the work, using a dilution of the same specimen of fluidextract in both chambers, it was found that if the same degree of dilution was reached in the two chambers the reductions were equal. If, however, unequal amounts were used, it often happened that the strip treated with the larger amount of ergot gave the lesser reduction. This was especially true if the epinephrine were added without previously washing out the ergot. Since the same ergot was used in both chambers, the conclusion seemed fair that there was something in it that was stimulant to smooth muscle and added to the epinephrine effect. Since this augmentation did not occur when solutions of the alkaloids were used, it could scarcely be ascribed to them when using the fluidextract. In view of the known presence of the actively oxytocic substance histamine in many ergot preparations, the effect of this substance on the epinephrine response was examined.

This question has been investigated before. Dale and Laidlaw (11) and Fröhlich and Pick (12) noted that histamine reduced the vasoconstrictor action of epinephrine. The latter authors also noted a reduction in the stimulant action of epinephrine on the pregnant uterus of the cat, *in situ*. Schegg found that synthetic ergot substitutes, made from histamine and tyramine reduced somewhat the reaction of the isolated uterus of the rabbit to epinephrine. Langecker (8), on the other hand, concerned lest histamine be a cause of inaccuracy in the assay of ergot preparations, found that histamine alone in concentrations between

1:100,000 and 1:1,000,000, did not alter the response of the rabbit's uterus to epinephrine.

Our own experiments suggest that histamine may alter considerably the action of epinephrine. We have observed repeatedly that if epinephrine be added in the presence of doses of histamine that are not of themselves stimulant, the form of the epinephrine curve is changed. Figure 1 shows such a phenomenon. This stimulation may occur to some extent even after the histamine is removed. In figure 2 are shown the results obtained when ergotamine alone was used on one strip and ergotamine plus histamine on the other. Even after one washing the strip to which the histamine had been added gives a greater contraction to epinephrine than does the one which did not have any histamine added to it. The second contractions, however, after two washings are practically identical. From results such as these we have drawn the conclusion that it is necessary to wash several times when making assays of galenical preparations. When comparing alkaloid solutions, this does not seem to be necessary. Using this modification, we have found that unknowns made up by one of us could be determined by the other usually with an accuracy of plus or minus 10 per cent.

### IV. COMPARATIVE RESULTS OF ASSAYS BY THE TWO METHODS

In table 1 are given the results of assays of a number of preparations by both the U. S. P. and the Broom-Clark method. These preparations fall into four groups. Sample 1 is the Standard Fluidextract supplied through the courtesy of the Pharmacological Laboratory of the Bureau of Chemistry in Washington. Samples 2 to 4 are fluidextracts made by us according to the U. S. P. from specimens of ergot obtained from various sources. Samples 5 to 9 are commercial fluidextracts obtained on the open market, all in the original containers except no. 6, which came from a druggist's stock bottle which had been on his shelves for at least six months, and opened at intervals during that time. Samples 10 to 13 are hypodermic preparations, all of which are claimed to correspond to more that 1 gram of ergot for each cubic centimeter of finished product. These amounts

of ergot are indicated in the table. It will be recalled that 1 cc. of Standard Fluidextract corresponds to 1 gram of drug. One specimen of the solid extract, no. 14, was assayed. There is no way of determining to how much ergot this preparation corresponds.

The method of reporting values must be explained. The Standard Fluidextract is rated as 100 in the table. Other preparations are given in relation to it. It will be noted that a number

TABLE 1

| NUMBER | TYPE OF PREPARATION | CLAIMED CONCENTRATION | ASSAY VALUE BY | |
|---|---|---|---|---|
| | | | U. S. P. | Broom-Clark |
| | | | *per cent* | *per cent* |
| 1 | U. S. P. Standard no. 635 | | 100 | 100 |
| 2 | Laboratory Fluid extract | | 200 | 190 |
| 3 | Laboratory Fluid extract | | 150 | 175–200 |
| 4 | Laboratory Fluid extract | | 100 | 100 |
| 5 | Commercial Fluid extract | | Under 25 | 5–10 |
| 6 | Commercial Fluid extract | | 100 | 100 |
| 7 | Commercial Fluid extract | | 100 | 100 |
| 8 | Commercial Fluid extract | | 125 | 130 |
| 9 | Commercial Fluid extract | | 125 | |
| 10 | Ampoule Preparation | 1 cc. = 2 gm. ergot | Under 25 | Under 6 |
| 11 | Ampoule Preparation | 1 cc. = 2 gm. ergot | Under 12 | Under 2 |
| 12 | Ampoule Preparation | 1 cc. = 2.5 gm. ergot | Under 10 | Under 3 |
| 13 | Ampoule Preparation | 1 cc. = 4 gm. ergot | | Under 2 |
| 14 | Ergotin Bonjean | ? | Under 25 | Under 1 |

of preparations are recorded as being "under" such and such a value. This means that for no. 12, which is an ampoule preparation said to contain the activity of 2.5 grams ergot per cubic centimeter, 2.0 cc. per kilogram gave no discoloration of the cock's comb. Since this preparation is two and one-half times the concentration of the U. S. P. fluidextract, it should give a discoloration in a dose of 0.2 cc. per kilogram. Ten times this dose is ineffective and therefore its value is recorded as "under 10." Actually it may be entirely deficient in alkaloids. The other ampoule preparations are rated in the same manner. The solid extract was assumed to represent a concentration of four

times, and 5 grams were suspended in 50 per cent alcohol, and the whole diluted up to 20 cc. Doses of this dilution up to 2 cc. per kilogram in roosters gave no color change in the comb, and no dose gave any epinephrine reversal. From this explanation it will be understood that for none of the preparations nos. 11 to 15 inclusive were actual values determined. There is a limit to the amount of material that can be injected into the breast muscles of cocks, or added to the baths containing the uterine strips without introducing factors other than those which it is intended to measure.

Inspection of the table reveals that the preparations fall into two groups, active and inactive. The commercial fluid extracts which were standardized in the manufacturers' laboratories before distribution were found here on reëxamination to conform to the claims made for them. It should be stated that all of them except no. 5 were bought in the original containers, small bottles containing from 1 to 4 ounces. Sample 5 was obtained from a local druggist's stock bottle, which had been opened and used for dispensing for at least six months. The fluid extracts made in the laboratory by the official method were also active preparations. On the other hand of the four ampoule preparations, not a single one possessed more than a trace of activity by either method. This seems to us a very important finding. One cannot of course in fairness say that all ampoule preparations are inert on the basis of examination of these particular lots. On the other hand, the results here reported should make one suspicious of all such preparations unless they are shown by proper methods of examination to contain some important amount of alkaloids. Undoubtedly a part of the often heard complaint of the clinicians regarding the lack of dependability of ergot is based on the use of these inactive preparations.[3] The one specimen of solid extract examined was inert, as has also been noted by Schegg (13).

[3] Since the experimental work was completed we have had occasion to examine one ampoule preparation not included in the above table, and found that by the Broom-Clark method it was as active as the U. S. P. fluid extract. A second lot of ampoules of the same make as No. 10 but of a more recent serial number was found to contain distinctly less than 50 per cent of the claimed activity, by the Broom-Clark method.

## V. THE STRENGTH OF THE U. S. P. X STANDARD

The present pharmacopoeial standard is a purely arbitrary one, namely a composite fluidextract made from ten different lots of ergot conforming to the U. S. P. botanical description. Such a standard solution has been prepared by the Pharmacological Laboratory of the Bureau of Chemistry of the United States Department of Agriculture.[4] When throughout this paper the U. S. P. Standard Fluidextract is mentioned, reference to this preparation is intended.

The Research Laboratories of the Pharmaceutical Society of Great Britain have adopted ergotoxin phosphate as a standard.

TABLE 2

*The results of a series of comparisons of U. S. P. Standard Fluidextract and Ergotamine by the Broom-Clark method*

| | |
|---|---|
| 1 cc. U. S. P. Standard is less than | 0.6 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is less than | 0.55 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is less than | 0.50 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is equal to | 0.45 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is equal to | 0.45 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is greater than | 0.40 mgm. ergotamine base |
| 1 cc. U. S. P. Standard is greater than | 0.35 mgm. ergotamine base |

"Fluidextracts made by the U. S. P. process are considered satisfactory if they are equivalent in activity to a 0.1 per cent solution of ergotoxin phosphate. A variation is permitted up to plus or minus 25 per cent of this value" (7).

We have assayed the U. S. P. Standard Fluidextract in terms of ergotamine, calculated as base. Actually, both the tartrate and the methanesulphonate have been used. The results of an assay by the Broom-Clark method are given in table 2.

From this it is concluded that the U. S. P. Standard Fluidextract is approximately the equivalent of a 0.045 per cent solution of ergotamine base, or a 0.053 per cent solution of the tartrate. This is practically the same value as that found by the cock's comb method as reported earlier in the paper. In a short

[4] Under the present organization, the Pharmacological Laboratory is in the Food, Drug and Insecticide Administration of the Department of Agriculture.

series of injections using ergotoxin instead of ergotamine, it was found that the U. S. P. Standard was the equivalent of a 0.04 per cent solution of ergotoxin, making ergotoxin slightly but definitely stronger than ergotamine, by the cock's comb method.

TABLE 3

*Showing the strength of a number of specimens of ergot as given in the literature*

| NUMBER | SOURCE | AUTHORITY | ALKALOID CONTENT |
|---|---|---|---|
| | | | *per cent* |
| 1 | Portuguese | Burn and Ellis | 0.133 |
| 2 | Spanish | Burn and Ellis | 0.12 |
| 3 | Spanish | Burn and Ellis | 0.11 |
| 4 | Spanish | Burn and Ellis | 0.075 |
| 5 | Spanish | Burn and Ellis | 0.067 |
| 6 | Spanish | Burn and Ellis | 0.075 (14 years old) |
| 7 | Russian | Burn and Ellis | 0.005 |
| 8 | Russian | Burn and Ellis | 0.005 |
| 9 | Polish | Burn and Ellis | 0.03 |
| 10 | ? | Forst (19)* | 0.082 |
| 11 | German | Forst (19)* | 0.080 |
| 12 | ? | Forst (19)* | 0.058 |
| 13 | German | Forst (19)* | 0.02 |
| 14 | Hungarian | Forst (19)* | 0.012 |
| 15 | German | Forst (19)* | 0.008 |
| 16 | Portugese | Linnell and Randle | 0.20 |
| 17 | ? | Nelson and Pattee† no. 2 | 0.086 |
| 18 | ? | Nelson and Pattee† no. 3 | 0.078 |
| 19 | ? | Nelson and Pattee† no. 4 | 0.045 |

* The assays of Forst were made by the method of Masuda (3).

† The values of the preparations no. 5 to no. 9 inclusive of the present report are not included, since their strength may have been adjusted by the manufacturer.

We are not aware that there has been published any attempt at determination of the strength of the U. S. P. Standard. Edmunds and Hale in 1911 suggested that 2 cc. fluidextract should produce the same discoloration as 5 mgm. of ergotoxin phosphate. This would make the fluidextract the equivalent of a 0.25 per cent solution of ergotoxin phosphate, which is two and one-half

times the British Pharmaceutical Society Standard, or approximately as we shall show later five times what our determinations show the present U. S. P. Standard to be. According to Linnell and Randle (16) a 0.1 per cent solution of ergot alkaloids is the strongest that can be obtained by percolation with acidulated alcohol.

The exact relation between the U. S. P. and the British Pharmaceutical Society Standards depends on the relative strength of ergotamine and ergotoxin, which question is discussed in the next section. Assuming for the present that the two alkaloids are equal in activity, the standard set up by Burn and Ellis is approximately twice that of the U. S. P.

Just why the British Standard has been set at its present value we do not understand. We have collected some of the recent figures bearing on the strength of ergot in table 3. Those given by Burn and Ellis and those taken from our own assays are not for ergot itself, but for the fluidextract. However, the method of the U. S. P. is said to be an effective method of extraction of the alkaloids and to extract them in concentrations up to 0.1 per cent in the percolate if they are present in adequate amount in the crude drug (16). At any rate, it will be noted in the table that only four of the drugs have an alkaloid content as high as that set by the British Laboratory. In addition Gittinger and Munch report that of their 69 samples only 23 were more than 100 per cent of the U. S. P. Standard, which is as we have shown about half that of Burn and Ellis. Certainly at most only a part of this group can be considered as being as high as 200 per cent of the U. S. P. To put it differently 46, or 67 per cent of their series were not over half the British figure. We are convinced that the figure is too high, and that although the present U. S. P. Standard is an arbitrary one, chosen without reference to an absolute value, it does happen to have a strength that practically is much more likely of attainment.

## VI. THE RELATION BETWEEN ERGOTAMINE AND ERGOTOXIN

It was indicated in the previous section that by the cock's comb method 0.4 mgm. ergotoxin is equal to 0.45 mgm. ergota-

mine, both calculated as bases. Because most of our determinations have been made in terms of ergotamine while those of Burn and Ellis are given in terms of ergotoxin, we have felt it necessary to compare the two substances again, and have made a number of such comparisons by the epinephrine-reversal method.

It seems generally to be assumed that the two alkaloids are identical in their pharmacological action even though they differ chemically. Very few actual comparisons seem to have been made. In the first paper on the subject, Dale and Spiro (2) reported the pharmacological identity of the two substances. Clark and Broom (17), however, found that ergotoxin was only half as effective in producing the reversal of the epinephrine effect on the rabbit uterus. On the other hand, Burn and Ellis (15) state that

> 3.5 parts of ergotamine *bi*tartrate could be equated to 3.0 parts of ergotoxin phosphate, or that ergotamine bitartrate contained 85 per cent of the activity of ergotoxin phosphate.
>
> If a calculation be made from the formula of ergotamine bitartrate $C_{33}H_{35}N_5O_5 \cdot 2C_4H_6O_6$, it is found that assuming the absence of water of crystallization, the base constitutes 65.9 per cent of the molecule. Similarly in ergotoxin phosphate $C_{35}H_{41}N_5O_6 \cdot H_3PO_4 \cdot H_2O$ the base constitutes 84.4 per cent of the weight of the molecule. Consequently it would be expected that if the base has the same activity in each case, ergotamine bitartrate would contain 78 per cent of the activity of ergotoxin phosphate. The figure found by the biological method differs from this by less than 10 per cent, and may be taken as confirmation of the identity of activity of the two bases (7).

In a letter to the Pharmaceutical Journal and Pharmacist, Mr. A. C. Bentley, questioning the use of ergotoxin phosphate as a standard states that "if the standard is the tartrate, as is usually supplied, and not the bitartrate, then the stated greater activity of ergotoxin phosphate is found to be non-existent."

We do not understand the reason for this statement. If one uses the percentage figures given in the above quotation, $0.659 \times 3.5$ parts ergotamine base are equal to $0.844 \times 3.0$ parts ergotoxin base in activity, or 2.3 parts ergotamine are equal to 2.5 parts

ergotoxin. It is only when the formula for the *tartrate is* used, that this superiority of ergotoxin appears. For then 0.845 × 3.5 ergotamine base[5] is equal to 0.844 × 3.0 ergotoxin base, or then ergotoxin clearly is the stronger of the two.

We have compared the two bases a number of times by the Broom-Clark method and have found repeatedly that ergotoxin is stronger than ergotamine, using either the methanesulphonate or the tartrate. The results of one such series of comparisons is given in table 4.

The ergotoxin used was a specimen of "amorphous ergotinine" from Merck obtained about 1925. The ergotamine was furnished

TABLE 4

*A comparison of ergotoxin and ergotamine by the Broom-Clark method*

| | | | |
|---|---|---|---|
| 1 cc. ergotoxin | is greater than | 1 cc. ergotamine | 2 determinations |
| 1 cc. ergotoxin | is equal to | 1 cc. ergotamine | 1 determination |
| 1 cc. ergotoxin | is greater than | 1.25 cc. ergotamine | 3 determinations |
| 1 cc. ergotoxin | is equal to | 1.33 cc. ergotamine | 1 determination |
| 1 cc. ergotoxin | is less than | 1.33 cc. ergotamine | 1 determination |
| 1 cc. ergotoxin | is greater than | 1.33 cc. ergotamine | 1 determination |
| 1 cc. ergotoxin | is less than | 1.5 cc. ergotamine | 1 determination |
| 1 cc. ergotoxin | is less than | 1.67 cc. ergotamine | 4 determinations |

through the courtesy of the Sandoz Company, in this case as the methanesulphonate, in tubes of about 20 mgm. Both substances were made up in terms of base, 1 mgm. per 25 cc. The ergotoxin was put into solution by the addition of a few crystals of tartaric acid. The solutions were kept in the refrigerator, and the assay was completed within 48 hours. The results are shown in table 4. From them it is assumed that 1 cc. of ergotoxin solution is the equivalent of 1.33 cc. of ergotamine solution, or that 1 mgm. of ergotoxin is equal in activity to 1.33 mgm. ergotamine. Expressed in the same way, Burn and Ellis' figures give 1 mgm. ergotoxin as the equivalent of 1.15 mgm. ergotamine (assuming that they were using the tartrate, as would appear from their introduction (7), p. 384).

[5] According to the Sandoz Chemical Works, the formula for ergotamine tartrate is, $2C_{33}H_{35}O_5N_5 \cdot 2CH_3OH \cdot C_4H_6O_6$; of which 84.5 per cent is base.

Having determined the relation between ergotoxin and ergotamine, the U. S. P. Fluidextract can be compared to the standard set up by the Research Laboratories of the Pharmaceutical Society of Great Britain. Their requirement that a fluidextract made by the U. S. P. method shall be the approximate equivalent of a 0.1 per cent solution of ergotoxin phosphate, would be a 0.084 per cent solution of ergotoxin base, or a 0.11 per cent of ergotamine base. Since we found by the U. S. P. method that our standard corresponded to a 0.05 per cent solution and by the Broom-Clark method a 0.045 per cent solution of ergotamine base, the U. S. P. Standard is actually slightly less than one-half that of Burn and Ellis.

## VII. CHOICE OF METHODS

It is our opinion as the result of making this series of comparisons that either method of assay can be used to determine the alkaloidal content of ergot preparations with reasonable assurance of obtaining accurate results. Both methods have certain difficulties of application, some of which have been indicated in the present paper. It is occasionally true for both methods, as is pointed out for the Broom-Clark method by Burn, that single determinations give results which differ widely from those ultimately found to be correct. Neither method can be used satisfactorily without reasonable familiarity with it. For work requiring only an occasional determination, or for chemical work which is being checked daily by biological methods, it seems to us that the Broom-Clark method offers definite advantages. It can be used at once, without the previous standardization of animals necessary by the other method. In can also be used to examine the activity of very dilute solutions of the alkaloids. On the other hand where a considerable number of assays have to be carried out routinely as in the laboratories of manufacturing pharmacists, it seems to us that the cock's comb method offers very distinct advantages. If a large number of birds is available, whose threshold has once been determined, then one person can made a number of assays in a single day. And certainly less general pharmacological training and experience is necessary

to use the method than is necessary to the successful use of isolated tissues. A certain amount of skepticism has been expressed regarding the accuracy of the U. S. P. method by various writers on the Broom-Clark method. It seems to us that the fact that we have examined these commercial fluid extracts by two methods and obtained results showing that the preparations conformed to a definite and published standard, can be taken as conclusive evidence that the manufacturers have been able to apply this method satisfactorily.

Reasons for believing that only methods of assay that determine the alkaloidal content have been reviewed in a recent paper by the authors (14).

## VIII. SUMMARY

The use of the U. S. P. X method of assay of ergot, and the epinephrine-reversal method introduced by Broom and Clark yields results practically identical. Both methods determine the alkaloidal content. By both methods the U. S. P. Standard Fluidextract is approximately the equivalent of a 0.05 per cent solution of the specific alkaloids, which is about one-half the value of the standard set up by the Research Laboratory of the British Pharmaceutical Association as suitable for fluidextracts. The British Standard is felt to be too high, since examination of the literature seems to indicate that very few specimens of ergot will yield a fluid extract measuring up to its value. It is the authors' opinion that a standard the equivalent of a 0.05 per cent solution of alkaloids would be more practical. Of the two alkaloids ergotoxin and ergotamine, the former is found to be slightly but distinctly stronger than ergotamine.

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