

able, were tested. Of these 6 were equal to or more active than the standard, and 16 were sub-standard. Perhaps some indication of country of origin may be obtained by comparison with the potencies of authenticated samples of ergot during the same season.

The Pharmacopœia states that ergot "deteriorates with age, and should not be kept longer than one year." It has been reported that climate, time of harvesting and the conditions of storage of crude ergot directly affect the quality. It may be that climatic conditions have been less favorable for Russian ergot than for Spanish. It is possible also that the samples from Russia are more than one year old.

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THE ASSAY OF ERGOT BY THE COCKSCOMB METHOD.

BY GEORGIANA SIMMONS GITTINGER, M.A. AND JAMES C. MUNCH, PH.D.

The method of assaying ergot, officially adopted in U. S. P. X is as follows:

"Ergot, in the form of the fluidextract, administered by intramuscular injection to single-comb, white Leghorn cocks, in doses not exceeding 0.5 cc. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of a standard fluidextract of ergot, * * * * *"

"Assay—Use single-comb, white Leghorn cocks, which are less than eighteen months of age, and weigh approximately 2 kilograms. Injections are made deeply into the breast muscles, and the effects are observed within one hour to one and a half hours after the administration of the drug. The same cock must not be used for testing purposes at shorter intervals than two weeks." (U. S. P. X, Ergota, page 133.)

The fluidextract of ergot is the form most commonly used in medicine. The solid extract, the powdered extract and various ergot pastes are unsuitable for assay as such, and are accordingly converted into fluidextracts for assay purposes. One part by weight of the paste or powder is dissolved in 3 parts of 50% ethyl alcohol to make a 1:4 solution.

A few experiments showed the same reaction of the comb whether the fluidextract was injected into the breast muscles or into the leg muscles. It is more practical to use the breast muscles, and the occasional development of local abscesses there is less serious than on the leg.

Various types of chickens were tested for possible substitution for the required white Leghorn cocks. Four rose double-comb white Leghorn cocks of suitable age, and weighing from 1900 Gm. to 2200 Gm. were tested at doses of 0.5 cc. and 1.0 cc. of U. S. P. X Standard per Kg. body weight. No effect was produced on either comb or wattles.

A series of hens was tested against a series of cocks of comparable weights; 90% of the cocks gave a satisfactory reaction, but the hens exhibited only slight blanching, or no effect whatever. Table I.

Breeds of cocks other than white Leghorns were tested also, with generally unfavorable results:

Brown Leghorns were more nearly satisfactory, but their combs returned to normal too slowly. They were also subject to loss of weight. Table II A.

Plymouth Rocks were tested, but too large a percentage of them failed to clear their combs within the specified two weeks (combs of white Leghorns usually clear in 24 to 48 hours); in addition, the birds at the proper age are too heavy. Table II B.

Rhode Island Reds of the right age which we used were too light in weight; and besides, their combs failed to clear within the two-week period. Table II C.

A code of numbers has been developed in this laboratory to denote the degree of darkening of the comb after injections. A satisfactory bluing of both lobe and tips of the comb, that meets standard requirements is recorded as 22. The figure

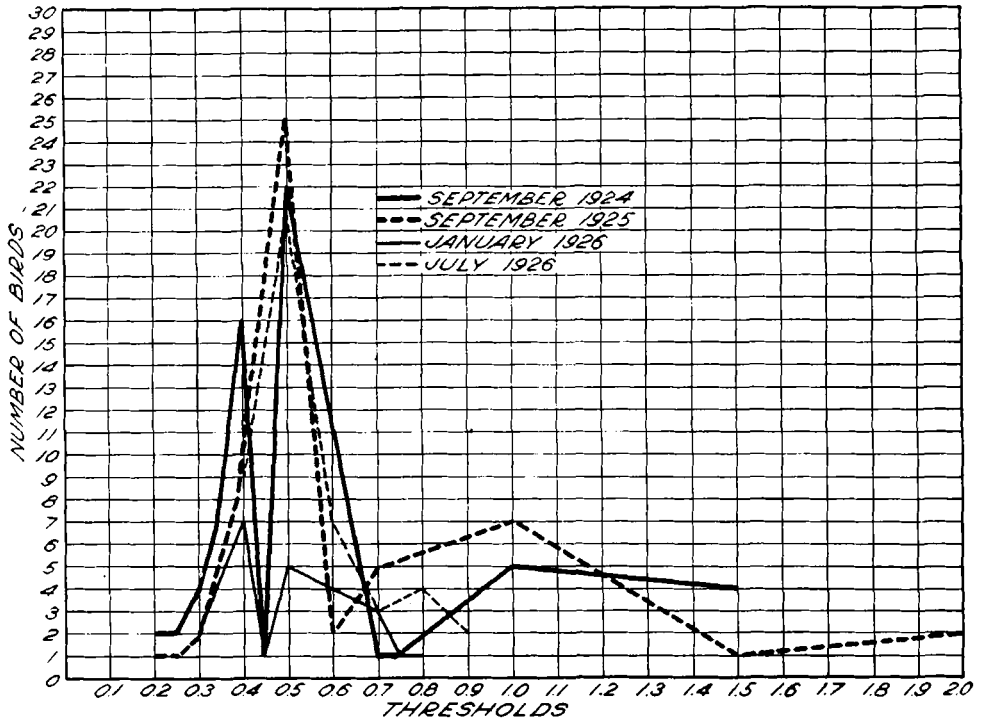


Chart I.

20 indicates that only the lobe is blue, and 21 that the lobe and first tip are the parts affected. A minimal effect is registered as 12 for lobe plus tips, 11 for lobe and one tip, 10 for lobe alone. The maximal effect, from too large a dose, is indicated by 33 for the whole comb, 32 and 31 for lobe and few or one tips, and 30 for lobe alone. This 30 is seldom encountered. Blanching of the comb in addition to the bluing is also recorded, and due consideration is given it in determining the potency of the sample being assayed.

Though the Pharmacopœia requires that "doses not exceeding 0.5 cc. for each Kg. of body weight of cock," shall produce the standard darkening of the comb, it has been found that the threshold dose—that dose which produces the just satisfactory bluing neither more nor less—varies with individual birds.

It is the practice in this laboratory to determine the threshold of a bird before using for assay purposes. The Standard Fluidextract of Ergot is used, and as many injections are made as may be necessary to determine the dose per Kg. which will give a satisfactory bluing of the comb. The majority of our birds have a threshold at 0.5 cc. per Kg.; but in our experiments we have had thresholds as high as 2.0 cc. per Kg. and as low as 0.2 cc. per Kg.

In four groups of birds obtained at different seasons, we found the variations in the threshold noted in Table III, and Graph I. It will be seen that in each group the highest percentage lies either at 0.5 cc. per Kg. or between 0.4 cc. and 0.5 cc. For the total of the several groups, the highest percentage of the whole 190 birds lies at 0.5 cc. per Kg. Table IV, Graph II.

When the threshold has been determined for a bird, the proper corrections are made in the final report on samples of Ergot assayed on him.

Thresholds may vary with age, sensitization or habituation to the drug. Therefore, at regular intervals the birds are reinjected at their previously determined threshold with doses of the U. S. P. X standard to verify that threshold. Very few alterations, whether more or less, have ever been found in it.

The most common variation is an increase in the threshold dose; the bird, perhaps, becoming habituated to the drug. However, when a threshold becomes increasingly higher the bird is discarded.

Birds are also discarded when their combs continually fail to return to normal within the rest period of two weeks.

Consideration is always given as to whether the bird has received a very high dose; recovery in such case, being naturally more retarded. Slower recovery is also noticeable after several months' constant use.

Observations of the effect of the drug are made from one hour to one and a half hours after injection. No change in darkening has been noted even after two-, or four-hour intervals. Though Edmunds finds that the color begins to fade after 2 hours,¹ our birds rarely clear their combs entirely under 24 hours. In

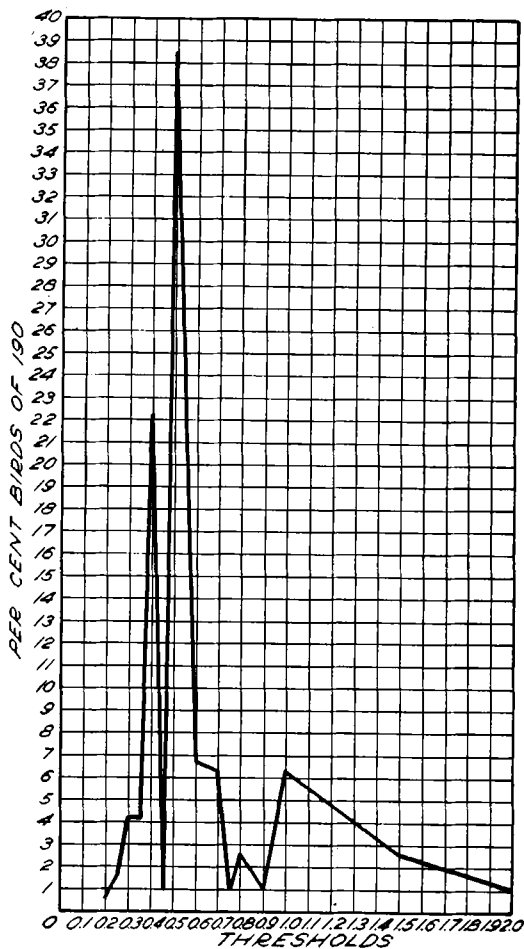


Chart II.

¹ C. W. Edmunds, *Hygienic Lab. Bull.*, No. 76, p. 25.

some cases, depending on the amount of the dose, they require 48 hours to return to normal.

The Pharmacopœia requires a two weeks' rest period. Edmunds reports using his birds after two to three days' interval, with no harmful effects. From results obtained in some special investigations we feel that a one-week interval is adequate for most birds; 10 days has been our shortest period, though in one experiment we used a bird which had made a speedy recovery, after four days, with good results. In no case was the bird injured or the accuracy of the method impaired.

Our method of assaying an unknown sample is to select three birds, giving to one a dose at his threshold, to a second at twice the threshold, and to the third at one-half the threshold. Further injections are then made on fresh birds at higher or lower per cent of the threshold as may be indicated by the previous results. For example, a sample may give no reaction at half, none at the threshold, and an insufficient effect at twice the threshold. Hence, the second injections would be given at twice once more, and at five times. However, should the first readings be maximal at all three doses, reinjections on fresh birds are made at one-half again and at one fourth and lower as may be indicated.

In case it is desired to ascertain the potency of a sample at a higher refinement of difference, successive injections are given of doses more closely spaced. After the potency of an unknown has been determined, we frequently inject several other birds for confirmation.

TABLE I.—INFLUENCE OF SEX ON REACTION.

Dose: 0.3 cc./Kg.				0.35 cc./Kg.				0.4 cc./Kg.				0.45 cc./Kg.			
Cock.		Hen.		Cock.		Hen.		Cock.		Hen.		Cock.		Hen.	
Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.	Wt.	Re-action.
1540	+	1310	-	1630	+	1510	-	1110	-	1060	-	..	-	1150	-
1280	+	1200	-	1360	+	1330	-	1050	+	920	-	..	-	1170	-
1630	+	1280	+
1350	+
1200	+

TABLE II.—INFLUENCE OF BREED ON REACTION.

A. Brown Leghorns.					
Cock no.	Weight.	Days since previous injection.	Dose.	Conditions of comb. On injection.	Final.
2291	1850	..	0.5	21
	1555	21	0.5	Blanched	21
	1400	11	0.5	33
	1305	18	0.3	Blanched	22
2463	1480	..	0.5	22
	1520	18	0.5	33
B. Plymouth Rocks.					
1919	3470	..	0.5	..	22
	3470	21	0.4	21	32
	3220	18	0.2	11	22
4186	3460	..	0.5	..	32
	2880	21	0.3	12	33
	2860	18	0.15	..	02

C. Rhode Island Reds.

2447	1250	..	0.25	..	32
	1365	13	0.125	10	20
	1550	13	0.15	..	33
3290	1500	25	0.13	11	21
	1640	..	1.0	..	33
	1435	13	0.5	10	33
	1520	25	0.3	11	32

TABLE III.—VARIATIONS IN THRESHOLD FOR 4 GROUPS OF WHITE LEGHORN COCKS.

Dose cc./Kg.	Number of birds.			
	Sept. 1924.	Sept. 1925.	Jan. 1926.	July 1926.
2.0	..	2
1.5	4	1
1.0	5*	7
0.9	2
0.8	1	4
0.75	1	..	1	..
0.7	1	5	3	3
0.6	..	2	4	7
0.5	22	25	5	21
0.45	1	..	1	..
0.4	16	10	7	9
0.35	8
0.3	4	2	2	..
0.25	2	1
0.2	..	1
	60% at 0.4-0.5	62% at 0.4-0.5	54% at 0.4-0.5	65% at 0.4-0.5

TABLE IV.—PER CENT OF EACH THRESHOLD DOSE ON TOTAL OF 4 GROUPS OF BIRDS.

Number of birds.	Dose cc./Kg.	% of total.	Number of birds.	Dose cc./Kg.	% of total.
2	2.0	1.0	73	0.5	38.4
5	1.5	2.6	2	0.45	1.0
12	1.0	6.3	42	0.4	22.1
2	0.9	1.0	8	0.35	4.2
5	0.8	2.6	8	0.3	4.2
2	0.75	1.0	3	0.25	1.6
12	0.7	6.3	1	0.2	0.5
13	0.6	6.8	—	—	—
			190		100.0

SUMMARY.

1. Ergot preparations should be converted into fluidextracts for assay.
2. Single-comb white Leghorn cocks have been found the most satisfactory breed of bird for assay purposes.
3. Variations in sensitivity to ergot are found in birds used for assay. Therefore all birds should be standardized before being used for the assay of unknown samples.
4. Durations of the effect of injection of the drug should be noted. Experiments under way suggest the possibility that the prescribed rest period of two weeks may safely be reduced to one week.

5. A sufficient number of assays should be made on any unknown sample to definitely determine its potency.

CONCLUSION.

By careful attention to details, ergot and its preparations may be satisfactorily assayed by the cockscomb method outlined in U. S. P. X.

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THE COLORIMETRIC ASSAY OF DIGITALIS.*

BY L. W. ROWE.

Several years have passed since Knudson and Dresbach (1) suggested the use of the Baljet (2) reaction for the colorimetric assay of digitalis preparations. Their work on about 30 preparations of digitalis indicates that the colorimetric method is about as accurate as the Hatcher cat method (3) and checks with the cat method very closely. Only two of the preparations were tested by the U. S. P. frog method. Kruse (4) has reported on the subject and finds the colorimetric method suitable for the determination of total activity but not for activity developed after absorption. He believes that the frog method is most suitable for the standardization of digitalis.

Although the M. L. D. frog heart method of Houghton (5) has been successfully used in our laboratory for standardizing digitalis preparations for more than thirty years, it seemed advisable to try out the colorimetric method because if it is sufficiently accurate it would reduce the time and expense of each assay.

Accordingly experiments were begun and direct comparisons were made between results obtained using the technique of Knudson with a Klett constant light colorimeter and those obtained at about the same time with the M. L. D. frog method. The first series was based on the Hatcher standard since it was known from previous comparisons that 8 heart tonic units of the frog test were about equal to 1 cat unit.

TABLE I.

Preparation and number.	Color test.	Frog test.	Color error.
Tr. Digitalis 768526	200%	105%	48% high
Tr. Digitalis 765576	220%	90%	60% high
Tr. Digitalis 766998	210%	90%	57% high
Tr. Digitalis 157585	200%	100%	50% high
Tr. Digitalis 767577	240%	110%	54% high
Tr. Digitalis 767577	250%	150%	40% high

(fortified)

In this series results were calculated from readings around 10 against 20 for the standard and also in some cases using 2 cc. of the sample instead of the usual 5 cc. It was found that results were particularly high by the color method when 2, 3 and 4 cc. of samples of tinctures were used with proportionate amounts of purifying reagents so this feature was abandoned.

* Scientific Section, A. Ph. A., Philadelphia meeting, 1926.