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TENTATIVE STANDARDS FOR SOME BIOLOGICALLY STANDARDIZED DRUGS.

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During the past four years we have had occasion to test by experiments upon lower animals a number of samples of drugs not suited for chemical assay. It seemed to us that an analysis of the results secured would possess a certain amount of interest and might shed some light on the unsettled question of biological assay. On the other hand, could it be assumed that the methods employed were reasonably accurate, inferences might be drawn as to provisional standards for some of these drugs. While no one will dispute the desirability of adjusting preparations of these drugs to definite standards, it must be admitted that two very important essentials have not yet been fulfilled to the satisfaction of everyone; namely, the selection of absolutely reliable assay methods and the clinical testing of preparations assayed by such methods. The methods we have employed have seemed to us the best, but it is quite possible that they are unsuitable, a point which can be settled only by careful investigation and cooperation among laboratory workers and between them and clinicians.

For the Digitalis series, we have used the one hour frog heart method of Cushny. Temperature is carefully noted and the end point is considered as reached when the frog's ventricle is motionless and tightly contracted in systole, sixty minutes after injection. The alcoholic strength of the solutions injected has varied between 5 and 33⅓ percent, a percentage as high as the latter not affecting the size of the dose necessary to stop the heart. The frogs have varied in weight from 10 to 40 grams, usually weighing near 20 grams. We have been unable to see any difference between male and female frogs in regard to resistance to poisoning by digitalis and its allies. At certain seasons, the frogs seem more susceptible to ouabain than at others while such is apparently not true of the susceptibility to digitalis. The values given below are the amounts in fractions of a gram per gram frog weight necessary to cause systolic stoppage of the frog's heart in 60 minutes.

First in the digitalis or heart tonic group comes convallaria. Three samples of convallaria root assayed at temperatures between 20° and 24° gave values of:

1. 0.000125
2. 0.000125
3. 0.000150

Two samples assayed at higher temperatures gave values of:

1. 0.0002 at 26°C
2. 0.00015 at 27°C

Three samples of F. E. Convallaria Root assayed at temperatures between 20° and 24° gave values of:

1. 0.0002
2. 0.000125
3. 0.000150

Average = 0.000158

Four samples of F. E. Convallaria Root assayed at higher temperatures gave values of:

1. 0.00015 at 25°
2. 0.0002 at 25°
3. 0.0001 at 27°
4. 0.0002 at 28°

Average = 0.000162

One sample of convallaria flowers assayed between 20° and 24° gave a value of 0.00005.

One sample of F. E. convallaria flowers assayed under similar conditions gave a value of 0.0001.

Twenty samples of digitalis leaf were assayed at temperatures between 20° and 24°. Of this number, two gave a value of 0.00035; three gave a value of 0.0004; six gave a value of 0.00045; two gave a value of 0.00050; four gave a value of 0.00055; one a value of 0.00060; one a value of 0.00065; and one a value of 0.00075.

Five samples of digitalis leaf were tested at higher temperatures with the following results:

1. 0.0004 at 25°
2. 0.00063 at 25°
3. 0.00045 at 25.5°
4. 0.00030 at 26°
5. 0.00045 at 27°

Nine Samples of F. E. digitalis were tested at temperatures between 20° and 24°. One sample gave a value of 0.00055; one, 0.00060; two, 0.00070; one, 0.00090; one, 0.00095; one, 0.0011; one, 0.0013; one, 0.0015. (Average 0.000922). Four samples of F. E. digitalis were tested at higher temperatures with results as follows:

1. 0.00085 at 26°
2. 0.00075 at 26.5°
3. 0.00070 at 29°
4. 0.00055 at 32.5°

Average = 0.000712

Eleven samples of tincture of digitalis were assayed at temperatures between 20° and 24°. One sample gave a value of 0.0050; three samples, 0.0060; five samples, 0.0065; one sample, 0.009; one sample, 0.010. (Average 0.0067.)

Three samples of tincture digitalis were tested at higher temperatures with the following results:

1. 0.005 at 26°
2. 0.0060 at 27°
3. 0.0055 at 29°

Average = 0.0055

Three samples of squill were assayed at temperatures between 20° and 24°. Of these three, two gave a value of 0.00045, one a value of 0.00050. Four samples of squill were assayed at higher temperatures, the results follow:

1. 0.0004 at 25°
2. 0.0005 at 26°
3. 0.0005 at 29°
4. 0.0005 at 29°

Twelve samples of F. E. squill, acetic, were assayed at temperatures between 20° and 24°. Of these, three gave a value of 0.0015; three a value of 0.00175; one a value of 0.002; one a value of 0.0022; two a value of 0.0025; two a value of 0.003.

Five samples of F. E. squill acetic tested at higher temperatures gave the following results:

1. 0.0035 at 25°
2. 0.0015 at 29°
3. 0.0040 at 30°
4. 0.0015 at 30.5°
5. 0.0035 at 31.5°

Three samples of strophanthus seed were tested at temperatures between 20° and 24°. Of these, one gave a value of 0.000006; one a value of 0.000015; one a value of 0.000010.

Five samples of strophanthus seed were tested at other temperatures, the results being:

1. 0.000006 at 18°
2. 0.000006 at 18.5°
3. 0.000005 at 25°
4. 0.000003 at 25°
5. 0.000003 at 33°

Twelve samples of tincture of strophanthus were tested at temperatures between 20° and 24°. Of these, one gave a value of 0.00004; three a value of 0.00045; one a value of 0.00052; one a value of 0.000055; two a value of 0.000060; one a value of 0.000080; one a value of 0.000085; one a value of 0.0001; and one a value of 0.00011.

Eight samples examined at higher temperatures gave values as follows:

1. 0.00017 at 24.5°
2. 0.000045 at 25°
3. 0.000050 at 25.5°
4. 0.000038 at 28°
5. 0.000045 at 29°
6. 0.000038 at 32°
7. 0.00004 at 28.5°
8. 0.00004 at 33°

All samples of crude drugs are examined by the botanist before being sent to our department, so that adulterated, mouldy, or otherwise unsatisfactory samples are not tested by us. It is immediately evident that a rather remarkable uniformity in strength exists in most of the samples of crude drugs that we have examined. Thus, of three samples of convallaria root assayed at the "optimum temperature," the variation in strength was only about 16 2/3 percent. Two samples examined at higher temperatures should, theoretically, have been capable of stopping the heart when given in smaller dose, but such was not the case.

Of twenty samples of digitalis leaf, secured, chiefly from German and English jobbers, examined at temperatures between 20° and 24°, fifteen did not vary much more than 30 percent while the greatest difference between the samples was 114 percent.

Of five samples of digitalis leaf tested at higher temperatures, four agreed fairly closely in strength, the largest dose being 50 percent greater than the

smallest. Here, also, temperature did not seem to play a very important role. The twenty samples assayed at the optimum temperature averaged 0.000490, while those assayed at higher temperatures averaged 0.000446.

Of the three samples of squill assayed between 20° and 24° two gave the same value while one required a dose 11.1 percent greater. Of the four samples assayed at higher temperatures, the strongest was sufficient in a dose 20 percent smaller than the remaining three. Here, three samples assayed at the optimum temperature gave an average value of 0.000466, while the four assayed at higher temperatures gave a value of 0.000475.

No such uniform results were secured in testing the samples of strophanthus seed submitted to us. Of the three samples tested at the optimum temperature, the extremes showed a difference of almost 300 percent. The samples tested at slightly lower temperatures gave the same values, agreeing with the best one of the three preceding. Of the three samples tested at higher temperatures, all were efficient in doses smaller than those found necessary at the optimum temperature, two of the samples being efficient in a dose one-fifth that of the smallest dose encountered when the testing was done at the optimum temperature. The average of the five samples tested at temperatures blow 24° was 0.0000086, while the three tested at higher temperatures gave an average value of 0.00000366. Apparently, temperature plays a much more important role in regard to strophanthus than it does in regard to the other members of the digitalis series tested, a point already brought out by Baker (Am. Jour. Pharm. Vol. 84, page 247, 1912).

Several interesting points seem to be evident from the tests of galenical preparations of these drugs. If first the results secured in assaying fluidextracts of convallaria, digitalis, and squill, are examined, it is seen that much greater variation is encountered than was the case in the examination of the crude drugs. Thus in three samples of F. E. convallaria tested at the optimum temperature the variation amounted to 60 percent; while in four samples tested at higher temperatures the variation was 100 percent. In nine samples of F. E. digitalis tested between 20° and 24°, the variation was almost 200 percent., while in four samples tested at higher temperatures the variation was about 54 percent.

In twelve samples of F. E. squill, acetic, assayed between 20° and 24°, there was a variation of 100 percent; while in five samples tested at higher temperatures, the variation amounted to almost 300 percent.

It is with considerable hesitancy that we draw positive conclusions from work carried out as ours has been, but while such uniform results were secured in testing samples of convallaria, digitalis and squill and such variations occurred when it was a question of the fluidextracts, it does seem at least to suggest that something is wrong with the fluidextracts, either the strength of menstruum or method of manufacture.

Houghton has already shown the practical impossibility of producing a satisfactory U. S. P. F. E. Digitalis.

Tr. of Digitalis, U. S. P., is, according to our tests, a more reliable preparation. Of eleven samples tested between 20° and 24°, eight agreed closely in

strength, the variation being only about 8 percent. Of three samples of tincture of digitalis tested at higher temperatures, the variation amounted to 20 percent.

Not only is there lack of uniformity in regard to the strength of these fluidextracts, but both the official fluidextracts and tinctures do not regularly represent the theoretical drug strength. Thus, five samples of convallaria root tested by us gave an average value of 0.000150, while seven samples of fluidextract of convallaria gave an average value of 0.000160.

Twenty-five samples of digitalis leaf gave a value of 0.000481, while thirteen samples of the fluidextract of digitalis gave an average of 0.000850. Seven samples of squill gave an average value of 0.00047, while twelve samples of acetic fluidextract of squill gave an average value of 0.002. The average of the value of fourteen samples of tincture of digitalis was 0.0065 against the average value 0.000481 found on testing the crude drug.

Of scarcely less importance than the digitalis group is ergot. Unfortunately, the work we have done in testing samples of ergot has not been so thorough as the preceding, so any conclusions drawn from the results secured are even more liable to be erroneous.

The method for testing ergot has been the one used for a number of years by Dr. Edmunds, and is a modification of Houghton's. The test animals are white Leghorn cocks of nearly a year old. It is desirable that the fowls be of practically the same age, weight, and kept under similar conditions. The drug, in the form of a fluidextract, in injected deep into the breast muscles, and changes in the comb are noted. The dose administered is considered efficient when distinct bluing of comb and wattles result within one or two hours.

In a number of instances we have run, in addition, blood-pressure experiments, according to the directions of Wood and Hofer, but these results possess, we believe, little importance in view of the work of Edmunds and Hale (Hygienic Lab. Bl. 76, July, 1911.), and our own experience, (Journ. A. Ph. A. Vol 1, p. 412, May, 1912).

Nineteen samples of ergot were tested by the cock's comb method. The first fifteen were administered in a dose of 1.5 gm. per kilo and caused bluing of the comb in that dose,[1] with only one exception, where a dose of 2 gm. per kilo was necessary. The last four samples caused bluing when given in a dose of 0.75 gm. per kilo, but failed to affect the comb when given in a dose of 0.50 gm. per kilo.

Of the nineteen samples, five were tested by the blood pressure method, the results being as follows:

	Initial rise in mm.	5 minutes after inj.	10 minutes after inj.	53 minutes after inj.
1	70	..	..	52
2	37	..	21	..
3	58	..	53	..
4	20	..	24	..
5	65	..	30	..

Fifty-six samples of fluidextract of ergot were tested by the cock's comb method. Of this number only two required a dose of 2 cc. per kilo, while three were efficient in a dose of slightly less than 0.75 cc. per kilo.

[1] No smaller doses were tried.

It might be inferred from these assays that carefully inspected ergot, free from adulterations and visible evidences of deterioration should cause bluing of the cock's comb in a dose of 1 to 1.5 gm. per kilo of body weight of white leghorn cocks as described. From the evidence we have gathered, we are inclined to believe that the official fluidextract of ergot is a good preparation, should run fairly uniform in strength, and should cause bluing of the comb when injected in a dose of 1 to 1.5 cc. per kilo.

So far as we can judge, the white leghorn cocks show no seasonal variation, but this is far from absolutely proven. Age affects their susceptibility to ergot, and individual fowls are encountered that possess increased or decreased susceptibility.

For cannabis indica we have employed Houghton's method, the drug being administered orally to dogs. It is important, we believe, to use one breed only, and fox terriers have seemed to us peculiarly suited for test animals. The method, at best, is crude, and uniform results can scarcely be expected when any mongrel is used.

Five samples of cannabis indica were tested. Two caused symptoms of intoxication in the dogs when given in a dose of 0.015 gm. per kilo; one produced symptoms in a dose of 0.018; one was efficient in a dose of 0.020; and one in a dose of 0.030. Four of these samples were tested near the same time in the years 1909, 1910 and 1912, so season played no part.

Fourteen samples of the fluidextract of cannabis indica were assayed with the following results:

1	0.015	8.	0.020
2	0.018	9.	0.020
3	0.018	10.	0.021
4	0.018	11.	0.024
5	0.018	12.	0.025
6	0.019	13.	0.050
7	0.020	14.	0.050

The last two samples assayed were made from deteriorated drug, so should not be considered. In the remaining twelve samples, the variation amounted to 66 2/3 percent.

It would seem that a standard requiring that 0.020 gm. of cannabis indica (or 0.020 cc. of a fluidextract of cannabis indica) per kilo of a suitable dog should not be a difficult one to conform to.

In conclusion we wish to emphasize the fact that the results we have secured are only suggestive. Were we dealing with definite substances of known strength, inferences as to the values of the methods would be justified, or if we were assaying substances of unknown strength by reliable and universally accepted methods, inferences could be safely drawn as to the probable strength of such substances. As it is, however, we are dealing with two unknowns. That such agreement as has been noted in some cases is the result of coincidence seems scarcely probable, however, and inclines us to place confidence in the methods and results.

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