SCIENTIFIC SECTION

A PRELIMINARY STUDY ON THE STANDARDIZATION AND STABILIZATION OF MYDRIATICS AND MYOTICS.

Paper V.

BY E. E. SWANSON, H. E. THOMPSON AND C. L. ROSE.

Munch[1] reported a bioassay method for the quantitative determination of mydriatics for use in assaying drug samples where chemical assays are unreliable. Munch and Gittinger[2] later reported a formula for calculating the composition of mixtures of mydriatic alkaloids. The outlined method as reported by Munch[1] was submitted to several collaborators. This report represents a study of their method by one of the collaborators. The writers have a further purpose in studying this method, and that is to apply it in determining the reliability of the chemical method for the standardization and stabilization of mydriatics. One of us (E. E. S.)[3,4,5,6] has reported the unreliability of the chemical methods for assaying aconite, gelsemium and veratrum as compared to the bioassay method, and also that the hydrogen-ion concentration controls the stability of these drugs, particularly aconite and veratrum. There has been some question as to the stability of U. S. P. preparations of belladonna, hyoscyamine and stramonium. These preparations are now under discussion. The chemical method U. S. P. and bioassay method (Munch) will be compared on the above U. S. P. preparations, and also with the hydrogen-ion concentration factor in regard to deterioration and stability. These results will be reported in a later article.

THE METHOD.

To briefly describe Munch's method: A cat is held one foot distant and directly in front of a 100-watt, nitrogen-filled, electric lamp to determine and compare the maximum contractibility of the two pupils under this condition. Five hundredths cc. of a neutral aqueous solution of a mydriatic is applied to the outer margin of one eye. The canthus is compressed and the lids opened and closed until the fluid disappears. At various intervals of time after the application of the mydriatic the cat is again subjected to the same light under the same conditions, and any differences in the diameter of the treated and of the untreated pupil are noted. A satisfactory reaction is produced when the pupil of the treated eye is just preceptibly wider than that of the untreated eye.

In determining the threshold doses of atropine sulphate, homotropine sulphate, ephedrine alkaloid and pseudoephedrine alkaloid, calculations were made in terms of the alkaloid per 1 liter. Ephedrine sulphate and pseudoephedrine

[1] *Jour. A. O. A. C.*, 10 (1927), 383.
[2] *Ibid.*, 11 (1928), 521.
[3] JOUR. A. PH. A., 12 (1923), 957.
[4] *Ibid.*, 13 (1924), 1108.
[5] *Ibid.*, 16 (1927), 296.
[6] *Ibid.*, 17 (1928), 23.

sulphate were calculated in terms of the salt. The minimum effective concentration was determined in mg. of the alkaloid or the salt per 1 liter; 0.05 cc. of the solution was used for each application. The readings were taken according to the following technique: Animal (cat) Number 14 in Table III is used as an example:

Atropine sulphate.	Mydriatic effect.	Pseudoephedrine alkaloid.	Mydriatic effect.
8 mg.	−	2500	−
10 mg.	+−	3000	+−
12 mg.	+	3500	+
14 mg.	++	4000	++

Homatropine sulphate.	Mydriatic effect.	Ephedrine sulphate.	Mydriatic effect.
80 mg.	−	30,000	−
100 mg.	+	40,000	+−
120 mg.	++	50,000	+
		60,000	++

Ephedrine alkaloid.	Mydriatic effect.	Pseudoephedrine	Mydriatic effect.
1500	−	60,000	−
2000	+−	70,000	+−
2500	+	80,000	+
3000	++	90,000	++

− = none.
+− = doubtful mydriatic action.
+ = positive effect or threshold dose.
++ = distinct effect.

DATA.

The following, Table I, represents the minimum effective concentration in mg. of alkaloid per liter, 0.05 cc. of the solution was used for each application. Twelve cats were used, each cat was given at least one test according to the above method. Some cats received four repeated tests:

TABLE I.

Cat no.	Atropine sulphate.	No. of tests.	Scopolamine hydrobromide.	No. of tests.	Hyoscyamine sulphate.	No. of tests.
1	12	3	0.3	2	4	3
2	8.6	3	0.3	2	5	4
3	10	3	0.2	2	4	5
4	10	3			4.2	4
5	12	3				
6	10	3	0.3	2	4.2	4
7	11.3	3	0.4	2	4.6	3
8	12	1			4.6	3
9	12	.			4	1
10	..	.	0.4	2	5.3	3
11	..	.	0.4	2	5	1
12	..	.	0.4	2	4	2
Total tests		22		16		33
Average	10.9		0.34		4.44	

The foregoing table shows that not all cats have the same threshold dose, but, if an average of all the threshold doses is taken, the figures are 10.9 mg. per liter for atropine, 0.34 mg. per liter for scopalmine, and 4.44 mg. per liter for hyoscyamine. A total of 22 tests were made on atropine, 16 tests on scopolamine, and 33 tests on hyoscyamine. Munch[1] reported 12 mg. per liter for the threshold dose for atropine, 0.4 mg. per liter for scopolamine, and 4 mg. per liter for hyoscyamine. He suggested, that in standardizing a cat, the animal should not be used unless it responds to a threshold dose of 12 mg. per liter of atropine. In this report the writers have not selected the cats in this way, but used them regardless of their threshold value. Considering the fact that the cats were not selected, the results agree favorably with those reported by Munch.

Table II represents the check assays of the above alkaloids on the same cats to determine the reliability of the method. This table shows the minimal effective concentrations in mg. of alkaloid per liter; 0.05 cc. of solution was used for each application.

TABLE II.

	Atropine Sulphate.			Scopolamine Hydrobromide.		Hyoscyamine Sulphate.			
Cat no.	1st assay.	2nd assay.	3rd assay.	1st assay.	2nd assay.	1st assay.	2nd assay.	3rd assay.	4th assay.
1	12	12	12	0.3	0.3	4	4	4	
2	8	8	10	0.3	0.3	5	5	4	5
3	10	10	10	0.2	0.2	4	4	5	4
4	10	10	10			5	4	4	4
5	12	12							
6	10	10	10	0.3	0.3	5	5	4	4
7	12	12	10	0.4	0.4	5	5	4	
8	12					4	5	5	
9	12					4			
10				0.4	0.4	5	6	5	
11				0.4	0.4				
12				0.4	0.4	4	4		

Table II shows that each cat had an approximate constant threshold value. Some cats checked in all tests and some did not vary more than is within the error of all biological methods of standardization.

TABLE III.

MINIMAL EFFECTIVE CONCENTRATION IN MG. OF ALKALOID PER LITER. 0.05 CC. OF SOLUTION WAS USED FOR EACH APPLICATION.

Cat no.	Atrop. sulph.	Homatrop. sulph.	Ephedr. alk.	Pseud. ephedr. alk.	Ephedr. sulph.	Pseud. ephedr. sulph.
13	10	80	2500	3500	40,000	70,000
14	12	100	2500	3500	50,000	80,000
15	10	80	2000	3000	60,000	70,000
16	12	100	3000	4000	60,000	80,000
17	14	120	3000	4000	60,000	90,000
18	12	120	3000	4000	60,000	90,000
19	12	100	2500	3500	50,000	80,000
20	12	100	2500	3500	50,000	80,000
Average	11.7	100	2650	3625	53,750	80,000

[1] *Jour. A. O. A. C.*, 10 (1927), 383.

Table III represents the threshold values of atropine sulphate, homatropine sulphate, ephedrine alkaloid and pseudoephedrine alkaloid in terms of the alkaloid. Ephedrine sulphate and pseudoephedrine sulphate are determined in terms of the salt.

The results in Table III show the average amount of alkaloid or salt of the alkaloid required as the threshold dose. This data correlates the results reported by Munch, in that the alkaloids ephedrine and pseudoephedrine are more active as mydriatics than their salts.

Finding the mydriatic method to be quantitative, this method was also used to determine the mydriatic values of five tinctures of Belladonna U. S. P.

TABLE IV.

Tr. no.	Year made.	Bioassay Method. (Munch) Atrop. alk. mg. per 100 cc.	Chemical Method. U. S. P. St. = 0.025 to 0.033.	p_H.
1	1924	0.0600	0.0300	5.97
2	1925	0.0410	0.0308	5.74
3	1926	0.2400	0.0300	5.97
4	1927	0.060	0.0298	5.60
5	1928	0.080	0.0286	5.77

These results show that by the cat-eye method the total amount of alkaloids is higher than is shown by the chemical method. This is probably due to the variability of the alkaloidal content of different lots of the crude drugs. Tincture No. 3, however, shows a discrepancy that is 8 times as active biologically as it is chemically. The other tinctures show approximately $1^1/_3$ to $2^1/_2$ times as active biologically as they do chemically. The increased bioassay results over the chemical results are probably due to the alkaloids, atropine, hyoscyamine and scopolamine. These alkaloids are found to vary in proportion in belladonna drug. This variability is not determined by the chemical method, which represents the total alkaloidal content as ether soluble alkaloids. By the cat-eye method scopolamine is approximately 30 times as active as atropine, hyoscyamine is about 3 times as active as atropine. Therefore, if the alkaloidal content of a certain lot of crude belladonna drug should contain more scopolamine and hyoscyamine than is usually found in the drug it would effect its mydriatic value or bioassay value more than the chemical assay. According to these results the chemical method is not a true method for determining the physiological effects of belladonna. The bioassay method and chemical method do not correlate. The chemical method gave a consistent quantitative assay of all five tinctures while the bioassay showed a variability of $1^1/_2$ to 8 times the activity. A series of tinctures of Belladonna U. S. P. are now being studied to determine more definitely this discrepancy between the chemical and bioassay methods, together with the hydrogen-ion concentration factor, and how it influences the standardization and stabilization of these preparations. The above p_H determinations are approximately the same for all the tinctures.

Some experiments were made on the myotic alkaloids, pilocarpine, physostigmine and arecoline, but the minimum effective concentration which we determined for pilocarpine and arecoline did not correlate with those found by Munch.

Further investigation has been outlined to study (1) the calculation of the

mydriatic value of the alkaloids of belladonna, (2) the effect of different hydrogen-ion concentrations on the deterioration and stabilization of the belladonna preparations and (3) the assay of myotic alkaloids.

CONCLUSIONS.

1. A study of the bioassay method for testing drugs that have a mydriatic action is reported.

2. The mydriatics, atropine sulphate, homatropine sulphate, scopolamine hydrobromide, hyoscyamine sulphate, ephedrine alkaloid, pseudoephedrine alkaloid, ephedrine sulphate and pseudoephedrine sulphate have been studied, and our results correlate with those of Munch.

3. Tinctures of belladonna have been assayed by this method and seem to show no deterioration, but do not correlate with the chemical method. Further work is necessary to determine this discrepancy.

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A STUDY OF THE COMPOSITION OF SODIUM BISMUTH TARTRATE.*

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INTRODUCTION.

Although many compounds of bismuth with tartaric acid have been prepared very little has been accomplished towards establishing their composition and structure. The date at which bismuth tartrate compounds were first made seems to be about 1847. Since that time numerous other compounds of bismuth and tartaric acid have appeared whose compositions seem to vary widely, dependent upon the mode of preparation. A number of the earlier preparations are reviewed by Warren[1] in his paper entitled "The Composition of Some Complex Bismuth Tartrates Used in the Treatment of Syphilis." In this paper Warren gives the results of his examination of several commercial specimens of bismuth tartrate. The results of his analyses for bismuth, tartaric acid and sodium or potassium content of the various samples are then compared with the theoretical composition of the compound as claimed by the manufacturers. According to Warren only one of the specimens examined proved to have the composition claimed by the manufacturers. This sample was believed to have the composition approximately represented by the formula:

$$\begin{matrix}\text{CH(OBiO)—COOK}\\ |\\ \text{CH(OBiO)— COOBiO}\end{matrix} + 4H_2O$$

Warren's examination confirmed this view.

It may be noted that in the analysis of this sample, Warren did not add ammonia water to bring the sample into solution, as he did with the other samples. The aqueous solution itself reacted alkaline to litmus.

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* Contribution from the Cobb Chemical Laboratory of the University of Virginia, No. 54.

[1] JOUR. A. PH. A., 14 (1925), 478-487.