There is very good agreement between the p_H as calculated from the solubility product and the p_H of purified Milk of Magnesia which had originally contained a slight excess of magnesium salt. We are unable to explain the value obtained by washing out an excess of caustic soda and this matter is being further investigated.

The electrometric method is more delicate than the U. S. P. titration method for free caustic, and we believe that the U. S. P. standard should be based on the p_H of the Milk of Magnesia rather than on any titration value.

LABORATORY OF E. R. SQUIBB & SONS,
NEW YORK.

THE STANDARDIZATION AND STABILIZATION OF ACONITE PREPARATIONS.*

PAPER I.

BY E. E. SWANSON AND A. L. WALTERS.

I. INTRODUCTION.

The standardization of aconite preparations by the present official chemical method U. S. P. IX Revision has been found to be unreliable. Rudolph and Cole,[1] Robinson,[2] Haskell[3,4,5], Haskell and Zirkle,[6] and others found considerable variation and inconsistency in the potency of aconite preparations, when assayed chemically and biologically. Aconite contains several alkaloids of variable pharmacological action and toxicity, but similar in chemical properties toward solvents and precipitants. Therefore the chemical method indicates the total ether-soluble alkaloids, including aconitine, which is regarded pharmacologically and therapeutically as the important alkaloid. Considering aconitine as the active therapeutic principle of aconite, and noting its high toxicity in comparison to the other alkaloids, particularly benzoylaconine and aconine, it seems to the writers that a lethal dose assay would furnish a method for testing the therapeutic efficiency of aconite, and that aconitine crystals could be used as a standard.

II. METHOD OF ASSAY.

Such a method has been developed by the Scientific Section of the American Drug Manufacturers' Association in which the writers have had a part. The method and its results have been published by Dohme,[7] Chairman Committee on Aconite of the Scientific Section of the American Drug Manufacturers' Association.

* Scientific Section, A. Ph. A., Asheville meeting, 1923.

This Committee sent out a sample of crystalline aconitine and a sample of Fluidextract of Aconite U. S. P. to the biochemists of five different laboratories. The object of this was to determine whether the results of a biochemical method would be sufficiently accurate and consistent in the various laboratories to justify its use as a method of assay.

Following are the results of this work:

	Aconitine cryst. per gram weight of guinea pig.	Fluidextract of aconite root.
Sharp & Dohme	0.0000000625	0.0000300
Upjohn	0.0000000600	0.0000266
Parke, Davis & Co	0.0000000600	0.0000300
Eli Lilly & Co	0.0000000625	0.0000275
Norwich Pharmacal Co	0.0000000510	0.0000360

The method of assay used by the various laboratories is as follows:

To assay the aconitine, dissolve 0.1 Gm. of the alkaloid in 100 cc of 2% acetic acid. Dilute 1 cc of this solution to 10 cc with distilled water giving a (1–10,000) solution of aconitine. Guinea pigs of 300 Gm. to 400 Gm. weight are used. Calculate the total dose required for each pig and dilute with normal saline solution to a total of 1.5 cc. This is injected into the subcutaneous tissue of the abdomen. The lethal dose is determined as the smallest amount which would kill within 24 hrs.

III. COMPARISON OF BIOCHEMICAL AND CHEMICAL ASSAYS.

The following data give a comparison between the chemical and biological assays of aconite root, fluidextract and tincture.

Aconite root.		Fluidextract aconite root.		Tincture aconite root.	
Biological assay standard 0.000040.	Chemical assay standard 0.50.	Biological assay standard 0.000040.	Chemical assay standard 0.50.	Biological assay standard 0.00040.	Chemical assay standard 0.050.
0.000030	0.50	0.000030	0.53	0.00027	0.052
0.000045	0.42	0.000040	0.46	0.00040	0.051
0.000035	0.37	0.000030	0.47	0.00035	0.048
0.000040	0.36	0.000030	0.50	0.00030	0.051
0.000030	0.43	0.000065	0.48	0.00025	0.048
0.000042	0.36	0.000025	0.47	0.00045	0.050
0.000040	0.67	0.000040	0.52	0.00045	0.065
0.000050	0.40	0.000040	0.54	0.00075	0.050
		0.000025	0.50	0.00040	0.055
Av. 0.000039	Av. 0.44	0.000030	0.47	0.00035	0.052
		0.000025	0.53	0.00045	0.049
		0.000032	0.53	0.00040	0.056
		0.000028	0.53	0.00050	0.047
		0.000028	0.53	0.00035	0.052
				0.00035	0.049
		Av. 0.000033	Av. 0.50	0.00035	0.050
				0.00030	0.049
				0.00020	0.051
				0.00040	0.055
				Av. 0.00038	Av. 0.051

(Guinea pigs weighed 300 Gm. to 400 Gm.)

The above data show a distinct inconsistency of the two methods.

For the fluidextract of aconite, dilute 1 cc to 10 cc with 50% alcohol. The same weight pigs are used and the calculations and dilutions with saline solution are the same as above.

For the tincture of aconite no dilutions are made except when each calculated dose is diluted with saline solution to 1.5 cc.

This biochemical method has been used in all the experiments here presented.

IV. SEASONAL VARIATION.

As in other biochemical testing, seasonal variation or resistance is considered an important factor in standardizing aconite preparations. To determine the importance of this factor, fresh solutions of the same sample of aconitine crystals were prepared each month for a year and tested. Results follow:

Date.	Lethal dose. Gm. per Gm. wt. of pig.
11–18–20	0.0000000625
12–19–20	0.000000060
1–11–21	0.000000065
2–22–21	0.000000070
3–23–21	0.000000065
4–5–21	0.0000000625
5–23–21	0.000000065
6–20–21	0.000000060
7–20–21	0.000000055
8–23–21	0.000000065
9–23–21	0.000000065
10–18–21	0.0000000575
11–20–21	0.000000060
2–20–22	0.000000065
1–5–23	0.000000050

(The pigs used 1–5–23 weighed only 250 Gm. to 300 Gm.)

These results show that the resistance of guinea pigs does not vary greatly, providing a standard weight of pig is used, and new lots of pigs are first kept in the laboratory for a month, so as to eliminate all source of error that may be due to diet and surroundings.

V. DETERIORATION OF ACONITE PREPARATIONS.

Even though the biochemical method gives a more accurate and efficient method of assay than the chemical one, the question of deterioration still is a problem of some concern. The deterioration of aconite preparations cannot be definitely determined until a satisfactory method of assay has been developed. However, it has long been known that there is a deterioration in the U. S. P. tincture and fluidextract of aconite, and the following data are a part of the experiments on deterioration.

A. Tincture of Aconite U. S. P.

Two samples of Tincture of Aconite U. S. P. (one assayed high and the other low when made).

Tincture.	Made.	Bio-assay.	Chem. assay.	Bio-assay 2–15–20.
557726	1–26–17	0.00020	0.057	Less than 13%
165850	11–14–18	0.00075	0.030	Less than 10% (no dose determined)

B. Fluidextract Aconite U. S. P.

Three samples of Fluidextract Aconite U. S. P. (one assayed high and two low).

Fluidextract.	Made.	Bio-assay.	Chem. assay.	Bio-assay 2-15-20.	Per cent. original bio-assay.
No. 618247	1-26-19	0.000065	0.48	0.000080	80
No. 600681	4-23-18	0.000025	0.47	0.000090	29
No. 571499	4-10-17	0.000025	0.56	0.000150	17

The above data show a very pronounced deterioration of the fluidextract and almost total loss of potency of the tincture in 1 to 3 years, and that the loss is gradual from year to year.

C. Aconitine Crystals.

The alkaloids of aconite are known to be rather unstable and easily hydrolyzed in dilute alcohol. Assuming that the salts of these alkaloids in solution are more stable and that the presence of some acid in the menstruum might prevent decomposition or deterioration, the writers made tests using the alkaloid aconitine, as follows:

Aging tests—aconitine crystals.

1. Prepared in ampuls on 11-17-20 a solution of aconitine (1-1000) in 70% alcohol or the same menstruum as the official tincture and fluidextract of aconite. When assayed was diluted (1-10), finishing (1-10,000).

DATA:

11-18-20 assayed 0.00000010 Gm. per Gm. weight
11-20-21 assayed 0.0000010 Gm. per Gm. weight
1-5-23 assayed 0.000010 Gm. per Gm. weight

Distinct loss in activity of the solution.

NOTE.—This assays only 60% as much when made as when 2% acetic acid is added.

2. Prepared in ampuls a solution of aconitine (1-1000) in 70% alcohol plus 2% acetic acid. This solution when injected was diluted (1-10), finishing (1-10,000).

DATA:

11-18-20 assayed 0.0000000625 Gm. per Gm. weight
11-20-21 assayed 0.0000000625 Gm. per Gm. weight
3-20-22 assayed 0.0000000575 Gm. per Gm. weight
1-5-23 assayed 0.000000055 Gm. per Gm. weight

(Pigs used 1-5-23 weighed only 250 Gm. to 300 Gm.)

There was no deterioration in three years.

3. Prepared in ampuls a solution of aconitine (1-1000) in 2% acetic acid solution. This solution when tested was diluted (1-10) making a (1-10,000) solution.

DATA:

11-18-20 assayed 0.0000000625 Gm. per Gm. weight
5-23-21 assayed 0.0000000575 Gm. per Gm. weight
10-18-21 assayed 0.0000000625 Gm. per Gm. weight
3-20-22 assayed 0.000000055 Gm. per Gm. weight
1-5-23 assayed 0.000000045 Gm. per Gm. weight

(Pigs used 1-5-23 weighed only 250 Gm. to 300 Gm.)

There was no deterioration in three years.

D. Experimental Preparations.

Deterioration experiments were made on tinctures of aconite manufactured from two lots of drug which assayed as follows:

Aconite Root No. 17048—8-18-20 assayed 0.000030 Gm.
Aconite Root No. 22040—9-27-20 assayed 0.000015 Gm.

Prepared three tinctures from each lot of drug as follows:

Series A.

Prepared on 11-20-20 three tinctures from drug No. 17048.

Tincture No. 1.—Menstruum 70% alcohol U. S. P.
Tincture No. 2.—Menstruum 70% alcohol, then 2% acetic acid added after percolation.
Tincture No. 3.—Menstruum 70% alcohol plus 2% acetic acid.

Series B.

Prepared 11-20-20 three tinctures from drug No. 20240.

Tincture No. 4.—Menstruum 70% alcohol U. S. P.
Tincture No. 5.—Menstruum 70% alcohol, 2% acetic acid added after percolation.
Tincture No. 6.—Menstruum 70% alcohol plus 2% acetic acid.

Series C.

Prepared 6-12-21 two tinctures.

Tincture No. 7.—Menstruum 70% alcohol U. S. P.
Tincture No. 8.—Menstruum 70% alcohol and 0.1% HCl added after percolation.

Following are the results of the experiments:

SERIES A.

Tincture No.	Date.	Bio-assay.	Chem. assay.	Standard aconitine crystals.	Corrected dose.	Per cent. bio-standard.	Per cent. chem. standard.
1	11-20-20	0.000275	0.042	0.0000000625	0.00022	181	84
2	11-20-20	0.000275	0.042	0.0000000625	0.00022	181	84
3	11-20-20	0.00030	0.041	0.0000000625	0.00024	166	82
1	4-25-21	0.00055	0.0375	0.0000000625	0.00044	90	75
2	4-25-21	0.000325	0.0416	0.0000000625	0.00026	154	83.2
3	4-25-21	0.00030	0.0458	0.0000000625	0.00024	166	..
1	11-28-21	0.0035	0.037	0.000000060	0.0030	13	74
2	11-28-21	0.000575	0.0416	0.000000060	0.00048	83	83
3	11-28-21	0.0030	0.0458	0.000000060	0.00025	160	91.6
1	12-25-22	.....	....	0.000000050	.....	..	..
2	12-25-22	0.000425	....	0.000000050	0.000425	93	..
3	12-25-22	0.000275	....	0.000000050	0.000275	166	..

SERIES B.

4	11-20-20	0.00015	0.066	0.0000000625	0.00012	333	132
5	11-20-20	0.00015	0.066	0.0000000625	0.00012	333	132
6	11-20-20	0.00014	0.079	0.0000000625	0.000112	335	158
4	4-25-21	0.00020	0.66	0.0000000625	0.00016	280	132
5	4-25-21	0.00015	0.72	0.0000000625	0.00012	333	144
6	4-25-21	0.00014	0.078	0.0000000625	0.000112	335	156
4	11-18-21	0.0011	0.0664	0.000000060	0.000916	43.6	132.8
5	11-18-21	0.00018	0.072	0.000000060	0.00015	266	144
6	11-18-21	0.00015	0.078	0.000000060	0.000125	330	156
4	12-25-22	.....	....	0.000000050	.....	...	..
5	12-25-22	0.00015	....	0.000000050	0.00015	266	..
6	12-25-22	0.00012	....	0.000000050	0.00012	333	..

SERIES C.

Tincture No.	Date.	Bio-assay.	Chem. assay.	Standard aconitine crystals.	Corrected dose.	Per cent. bio.-standard.	Per cent. chem. standard.
7	6–12–21	0.000375	0.050	0.000000060	0.00312	128	100
8	6–12–21	0.000375	0.050	0.000000060	0.000312	128	100
7	11–18–21	0.0040	...	0.000000060	0.00333	12	..
8	11–18–21	0.00045	...	0.000000060	0.000375	106	..
7	10–12–22	.....	...	0.0000000575	.....	..	..
8	10–12–22	0.00041	...	0.0000000575	0.00035	114	..
7	12–25–22	.....	...	0.000000050	.....	..	..
8	12–25–22	0.00030	...	0.000000050	0.00035	114	..

To briefly summarize the above tables:

The tinctures Nos. 1, 4 and 7 prepared according to the United States Pharmacopœia shows a very rapid loss of potency in one year's time, as follows:

Tincture No.	Date.	Bio-assay. Per cent.	Chem. assay. Per cent.	Date.	Bio-assay. Per cent.	Chem. assay Per cent.
1	11–20–20	181	84	11–28–21	13	74
4	11–20–20	333	132	11–18–21	43.6	132.8
7	6–12–21	100	100	11–18–21	12	100

The chemical assay shows no loss of activity compared to the bio-assay.

The tinctures Nos. 2, 5 and 8 prepared according to the United States Pharmacopœia with 2% acetic acid or 0.1% hydrochloric acid added to the percolate or finished percolation, show some loss in potency, but not equal to the above experiments.

Tincture No.	Date.	Bio-assay. Per cent.	Chem. assay. Per cent.	Date.	Bio-assay. Per cent.	Chem. assay. Per cent.
3	11–20–20	181	84	12–25–22	93	..
5	11–20–20	333	132	12–25–22	266	..
8	16–12–21	128	100	12–25–22	114	..

These experiments show that the addition of acid to the percolate or finished percolate partially stabilizes the potency of the tincture.

Tinctures Nos. 3 and 6 prepared with a menstruum of 70% alcohol and 2% acetic acid completely prevents deterioration:

Tincture No.	Date.	Bio-assay. Per cent.	Chem. assay. Per cent.	Date.	Bio-assay. Per cent.	Chem. assay. Per cent.
3	11–20–20	166	82	12–25–22	166	..
6	11–20–20	335	158	12–25–22	333	..

No loss in activity in two years' time, and seems to permanently stabilize the potency of aconite tincture and fluidextract.

VI. CONCLUSIONS.

To briefly summarize the results obtained by the chemical method and guinea pig lethal dose method of assaying aconite and its preparations, it may be said that the chemical method is unreliable, and should be disregarded in the next revision of the U. S. P. The lethal dose assay has given much better results in the hands of various laboratories and technicians. The use of crystalline aconitine is a satisfactory standard when the same lot of crystals is used. The adoption of aconitine as a standard will necessitate a strict definition of the physical and chemical properties of such a standard.

The experiments reported on deterioration show conclusively that the deterioration of the tincture and fluidextract of aconite can be prevented by the addition of 2% acetic acid or 0.1% hydrochloric acid to the menstruum.

1. Rudolf & Cole, *Amer. J. M. S.*, 144, 788, 1912.
2. Robinson, *"The Archives of Internal Medicine,"* Vol. 15, p. 645.
3. Haskell, *Am. Jour. Pharm.*, 87, 489, 1915.
4. Haskell, *Am. Druggist & Pharm.*, Vol. 64, p. 129.
5. Haskell, *Am. Jour. Pharm.*, Vol. 88, p. 3, 1916.
6. Haskell and Zirkle, *Ibid.*, Vol. 87, p. 537.
7. Dohme, JOUR. A. PH. A., 10, 428, 1921.

LILLY RESEARCH LABORATORIES,
ELI LILLY & CO., INDIANAPOLIS.

A STUDY OF THE RELATIVE PRESERVATIVE VALUES OF GLYCERIN AND SUGAR SOLUTIONS IN CERTAIN OFFICIAL PREPARATIONS.

BY JOHN C. KRANTZ JR.

The question of preservation of medicinal salts with glycerin or sugar solutions has been the source of a large amount of investigation and intense study. The rapid deterioration of certain syrups, caused by either oxidation or hydrolysis, and the rapidity with which sucrose is inverted and often caramelized in certain galenicals is a constant source of annoyance to the pharmacist. The decomposition of practically all of the official syrups and solutions containing mineral substances may be divided into two classes: first, oxidation through atmospheric influence, and second, hydrolysis or decomposition caused by the ions of water.

PART I.

A. SYRUP OF FERROUS IODIDE.

As one of the type of preparations which readily decomposes under atmospheric influence, Syrup of Ferrous Iodide may be cited. Accordingly a syrup was prepared by the pharmacopœial process omitting the dilute hypophosphorous acid. At different periods after preparing the amount of invert sugar present was estimated by decomposing a small accurately weighed quantity of the syrup with sodium carbonate solution, removing the precipitated ferrous carbonate by filtration and estimating the invert sugar in the filtrate gravimetrically with Fehling's solution.

5 days showed.....	6.5%	invert sugar
15 days showed.....	7.5%	invert sugar
30 days showed.....	8.8%	invert sugar
75 days showed.....	10.96%	invert sugar

Another syrup was prepared including the dilute hypophosphorous acid and the following results show the inversion of the sucrose:

10 days showed.....	94.2%
20 days showed.....	96.2%

These results indicate that within a period of twenty days practically all of the sugar is inverted, whereas if the acid is omitted the degree of inversion is far less.