THE STANDARDIZATION AND STABILIZATION OF VERATRUM PREPARATIONS AND THE HYDROGEN-ION CONCENTRATION FACTOR.—PAPER VI.*

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I. INTRODUCTION.

In a number of previous articles (1, 2, 3, 4) the standardization and stabilization of aconite, nux vomica, gelsemium and veratrum preparations were reported. These reports include the study of a bio-assay method; the deterioration of preparations of aconite; the unreliability of the chemical method; the prevention of deterioration; the cause of deterioration or hydrolysis of the aconitine alkaloid into the less potent alkaloids, benzylaconine and aconine; and, finally, the hydrogen-ion concentration, which was shown to control the deterioration and stabilization. Swanson and Hargreaves (4) further reported that inasmuch as it was found that the above factors control the value of aconite preparations, other drug preparations were studied with the thought that possibly other fluidextracts and tinctures have a hydrogen-ion concentration or p_H value that might control their deterioration and stabilization. The drugs or fluidextracts of drugs that were studied are: Fluidextracts of Nux Vomica U. S. P. IX, Gelsemium U. S. P. IX and Veratrum U. S. P. IX.

It was found that the fluidextract of Nux Vomica U. S. P. IX is stable and does not require a definite hydrogen-ion concentration to control its potency; it does not deteriorate; the chemical method and bio-assay method gave correlative results. Fluidextracts of Gelsemium U. S. P. IX require no definite hydrogen-ion concentration to stabilize its alkaloids; the chemical method is unreliable; and this preparation should be assayed biologically.

The fluidextract of Veratrum U. S. P. IX seems to require some definite hydrogen-ion concentration to control its deterioration and stabilization. The chemical method is unreliable; this drug should be assayed biologically.

This report represents a more extensive study of the hydrogen-ion concentration factor in regard to the deterioration and stabilization of fluidextracts and tinctures of Veratrum U. S. P. IX. The chemistry of veratrum alkaloids has been studied by Eden (5). The pharmacological action has been reported by Pilcher and Sollman (6). The chemical assay of veratrum alkaloids, as found by several authorities, is not an indication of its therapeutic value. Houghton and Hamilton (7), reported the assay of a Veratrum preparation on frogs; later, Pilcher (8), reported a bio-assay method on frogs; and, still more recently, Rowe (9), described the use of mice as a bio-assay method.

II. EXPERIMENTAL DATA.

Two fluidextracts and one tincture of Veratrum were prepared. These preparations were so divided as to represent eight, ten or eleven samples. These samples received either no acid or different amounts of HCl 36% C.P. Each

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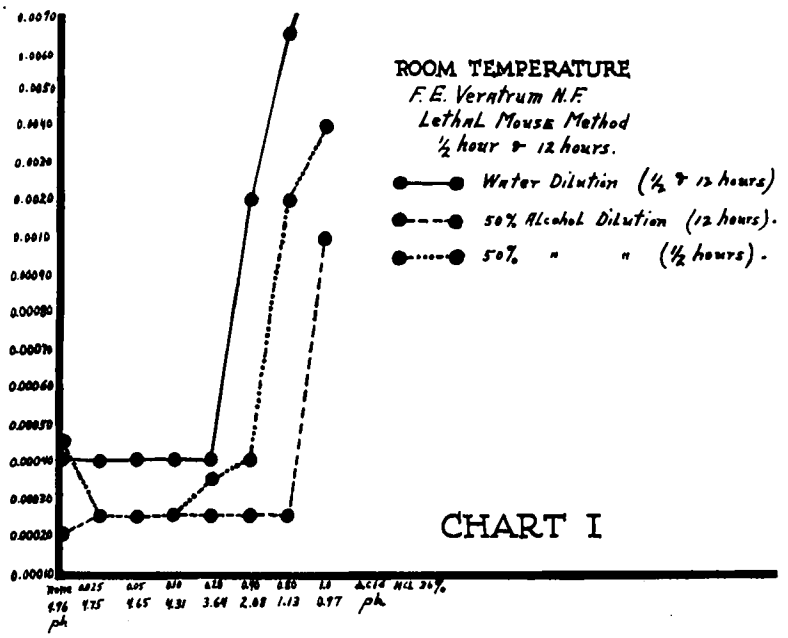
sample was assayed by the White Mouse Method as reported by Rowe, together with one-half-hour and twelve-hour lethal dose readings. Dilutions were made with distilled water and 50% alcohol. The hydrogen-ion concentrations were determined by the electrometric method. The difficulties in these determinations may be overcome by a method reported by Caldwell (10). Some of the samples were kept in an oven at 40° to 50° C. for seven months.

EXPLANATION OF TABLES.

Table I represents a fluidextract of Veratrum U. S. P. IX, made February 3, 1927, divided into eight parts. To seven of these were added various amounts of HCl 36%. All samples were kept at room temperature and exposed to light. The original assay was 0.00035 Gm. per



Gm. of mouse; they were all assayed again March 15, 1929, by the Lethal Mouse Method and one-half-hour and 12-hour readings were taken. Dilutions were made with distilled water and 50% alcohol. The hydrogen-ion concentrations were determined by the electrometric method.

TABLE I.—FLUIDEXTRACT OF VERATRUM U. S. P. IX. KEPT AT ROOM TEMPERATURE. MADE IN 2-3-27. TESTED 3-15-29. ORIGINAL ASSAY = 0.00040 GM. PER GM. (MOUSE). STANDARD DOSE = 0.00025 GM. PER GM. (MOUSE).

Sample number.	Am't. of 36% HCl in 100 cc.	p_H.	M. L. D. ½ hr. dose per Gm. H_2O.	M. L. D. 12 hrs. dose per Gm. H_2O.	M. L. D. ½ hr. dose per Gm. 50% alcohol.	M. L. D. 12 hrs. dose per Gm. 50% alcohol.	Bio-assay ½ hr. H_2O per cent.	Bio-assay ½ hr. 50% alcohol per cent.	Bio-assay 12 hrs. 50% alcohol, per cent.	Chemical assay per cent.
1	1%	0.97	0.0080	0.0080	0.0040	0.0010	3%	6.25%	25%	1.065%
2	0.8%	1.13	0.0065	0.0065	0.0020	0.0020	3.85%	12.5%	12.5%	.....
3	0.4%	2.08	0.0020	0.0020	0.00040	0.00025	12.5%	62.5%	100%	.....
4	0.2%	3.64	0.00040	0.00040	0.00035	0.00025	62.5%	71%	100%	.....
5	0.10%	4.31	0.00040	0.00040	0.00025	0.00025	62.5%	100%	100%	.....
6	0.05%	4.65	0.00040	0.00040	0.00025	0.00025	62.5%	100%	100%	.....
7	0.025%	4.75	0.00040	0.00040	0.00025	0.00025	62.5%	100%	100%	.....
8	None	4.96	0.00040	0.00040	0.00045	0.00025	62.5%	55%	100%	1.047%

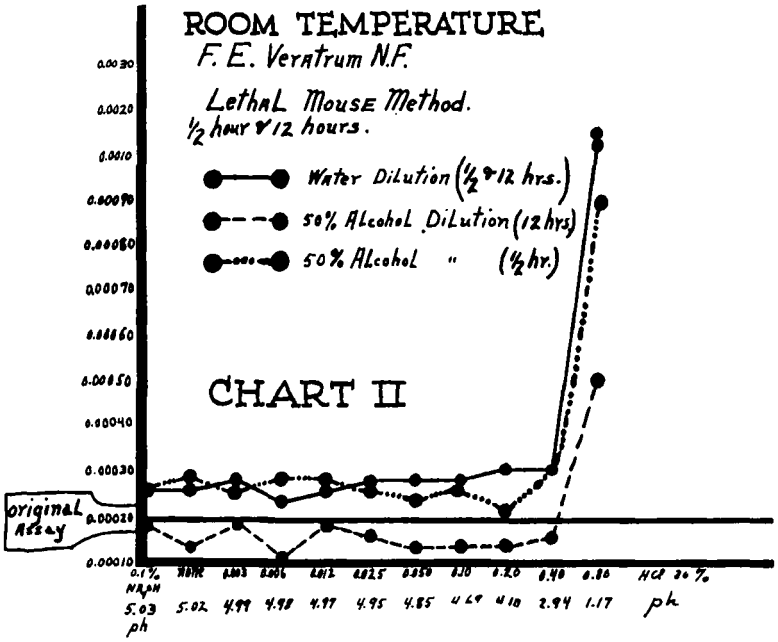
III. DISCUSSION.

The data in Table I show that this fluidextract of Veratrum U. S. P. IX maintained its potency for over two years with a p_H of 4.96 to 3.64. With a p_H of 2.08, 1.13 and 0.97 there is

considerable deterioration, the deterioration increasing as the p_H increases. With distilled water dilutions, the lethal dose is the same after one-half-hour and twelve-hour periods. Fifty per cent alcohol, however, increases the toxicity of the preparation for both the one-half-hour and twelve-hour periods.

This preparation having a p_H of 4.96 required no acid for maintaining its potency. Acid could be added to the preparation without injury, until a p_H of 3.64 was reached, and too much acid decreased its activity.

Table II represents a fluidextract of Veratrum U. S. P. IX that was made February 24, 1928. It was divided into eleven parts; to nine of these samples various amounts of HCl 36% were added. To one of the samples 0.1% NH_4OH 40% was added. All samples were kept at room temperature and exposed to light. The original assay was 0.00020 Gm. per Gm. of mouse. They were all assayed again May 20, 1929, by the Lethal Mouse Method. One-half-hour and



twelve-hour readings were taken. Solutions were made with distilled water and 50% alcohol. The hydrogen-ion concentrations were determined by the electrometric method.

TABLE II.—FLUIDEXTRACT OF VERATRUM N. F. KEPT AT ROOM TEMPERATURE. MADE IN 2-24-28. TESTED 5-20-29. ORIGINAL ASSAY = 0.00020 GM. PER GM. (MOUSE).

Sample number.	Am't. of 36% HCl in 100 cc.	p_H.	M. L. D. ½ hr. dose per Gm. H_2O.	M. L. D. 12 hrs. dose per Gm. H_2O.	M. L. D. ½ hr. dose per Gm. 50% alcohol.	M. L. D. 12 hrs. dose per Gm. 50% alcohol.	Bio-assay H_2O Per cent.	Bio-assay ½ hr. 50% alcohol per cent.	Bio-assay 12 hrs. 50% alcohol per cent.	Chemical assay
1	0.8%	1.17	0.00125	0.00125	0.00090	0.00050	20%	27.2%	50%	1.045
2	0.4%	2.94	0.00030	0.00030	0.000275	0.00015	83%	83%	166%	...
3	0.2%	4.10	0.000275	0.000275	0.00020	0.000125	91%	125%	200%	...
4	0.1%	4.69	0.000275	0.000275	0.00025	0.000125	91%	100%	200%	...
5	0.05%	4.85	0.000275	0.000275	0.000225	0.000125	91%	111%	200%	...
6	0.025%	4.95	0.00025	0.00025	0.00025	0.00015	100%	100%	166%	...
7	0.0125%	4.97	0.000225	0.000275	0.000275	0.000175	111%	83%	143%	...
8	0.0060%	4.98	0.000275	0.000275	0.000275	0.00010	91%	83%	250%	...
9	0.0030%	4.99	0.000250	0.000250	0.00025	0.000175	100%	100%	192%	...
10	None	5.02	0.000250	0.000250	0.000275	0.000125	100%	83%	200%	...
11	0.1% NH_4OH	5.03	0.000250	0.000250	0.00025	0.000125	100%	100%	200%	1.037

The data show that this fluidextract of Veratrum U. S. P. IX has not deteriorated. Dilutions made with 50% alcohol gave the same lethal dose as dilutions with distilled water in the one-half-hour period, but in the twelve-hour period or readings the toxicity increased below the original assay of the preparation.

This preparation required no acid to stabilize its activity. Acid could be added to the preparation without injury until a p_H of 4.10 was reached; too much acid decreased the activity. Dilutions made with distilled water and 50% alcohol gave correlative readings in one-half-hour periods. The toxicity of the preparation was increased by 50% alcohol dilutions during the twelve-hour period.

Table III treats of the same preparation as that of *Table II*. These samples, however, were kept at an incubation temperature of 50° C. and were not exposed to light for 7 months; the original assay before incubation was 0.00020 Gm. per Gm. of mouse. These samples were again assayed May 25, 1929, by the Lethal Mouse Method. One-half-hour and twelve-hour readings

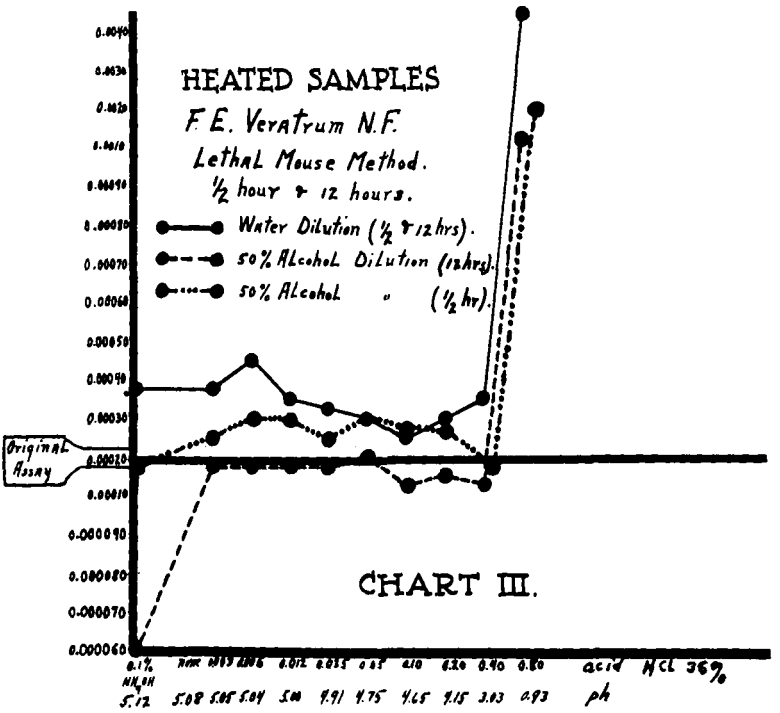


TABLE III.—FLUIDEXTRACT OF VERATRUM N. F. HEATED SAMPLES. MADE IN 2-24-28. TESTED 5-25-29. ORIGINAL ASSAY = 0.00020 GM. PER GM. (MOUSE). STANDARD M. L. D. = 0.00025 GM. PER GM. (MOUSE).

Sample number.	Am't. of 36% HCl in 100 cc.	p_H.	M. L. D. ½ hr. dose per Gm. H_2O.	M. L. D. 12 hrs. dose per Gm. H_2O.	M. L. D. ½ hr. dose per Gm. 50% alcohol.	M. L. D. 12 hrs. dose per Gm. 50% alcohol.	Bio-assay ½ hr. H_2O. per cent.	Bio-assay ½ hr. 50% alcohol per cent.	Bio-assay 12 hrs. 50% alcohol per cent.	Chemical assay, per cent.
1	0.8%	0.93	0.0045	0.0045	0.0020	0.00125	5.5%	12.5%	20%	1.045
2	0.4%	3.03	0.0035	0.0035	0.000175	0.000125	7%	1.42%	200%	...
3	0.2%	4.15	0.00030	0.00030	0.000275	0.00015	83%	91%	166%	...
4	0.10%	4.65	0.00025	0.00025	0.000275	0.000125	100%	91%	200%	...
5	0.05%	4.91	0.00030	0.00030	0.00030	0.00020	83%	83%	125%	...
6	0.025%	5.00	0.000325	0.000325	0.00025	0.000175	77%	100%	143%	...
7	0.0125%	5.04	0.00035	0.00035	0.00030	0.000175	71.5%	83%	143%	...
8	0.0060%	5.05	0.00035	0.00035	0.00030	0.000175	71.5%	83%	143%	...
9	0.0030%	5.08	0.000375	0.000375	0.00025	0.000175	66.6%	100%	143%	...
10	0.1% NH_4OH	5.12	0.000375	0.000375	0.000170	0.000060	66.6%	147%	417%	1.037

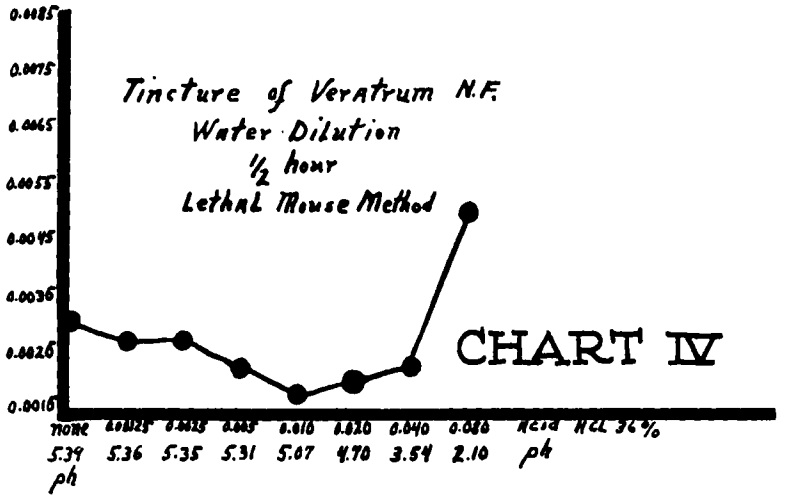
were taken. Dilutions were made with distilled water and 50% alcohol. The hydrogen-ion concentrations were determined by the electrometric method.

The data show that during the incubation period this preparation deteriorated more than did the preparations kept at room temperature. Sample No. 4 has the same potency as several of the most active samples in Table II. Dilutions with distilled water gave the same readings in the one-half-hour and twelve-hour periods. Fifty per cent alcohol dilutions in the one-half hour-period gave a higher potency than did the distilled water dilutions; 50% alcohol dilutions in the twelve-hour period show an increase in activity over the original assay.

Sample No. 10, containing NH_4OH, shows a toxicity of 0.000060 Gm. per Gm. of mouse in the twelve-hour period—a great increase in toxicity.

These samples, particularly the alcohol dilutions show considerable variation in activity. A p_H of 4.64 seems to stabilize the preparation equal to the preparation in Table II.

Table IV represents a tincture of Veratrum U. S. P. IX that was made February 27, 1928. This preparation was divided into eight parts; various amounts of HCl 36% were added. The samples were kept at an incubation temperature of 50° C. and were not exposed to light for seven months. The original assay was 0.0018 Gm. per Gm. weight of mouse. These samples were assayed again April 9, 1929. One-half-hour and twelve-hour lethal dose readings were taken.



Dilutions were made with distilled water. The hydrogen-ion concentrations were determined by the electrometric method.

TABLE IV.—TINCTURE OF VERATRUM N. F. HEATED SAMPLES (7 MONTHS). ORIGINAL ASSAY = 0.0018 GM. PER GM. (MOUSE). (TINCTURE MADE 2-27-28). TESTED 4-9-29.

Sample number.	Am't. of 36% HCl 100 cc.	p_H.	M. L. D. ½ hr. H_2O.	M. L. D. 12 hrs. H_2O.	Bio-assay per cent.	Chemical assay.
1	0.080%	2.10	0.0050	0.0050	50%	0.105
2	0.040%	3.54	0.00275	0.00275	91%	...
3	0.020%	4.70	0.0020	0.0020	125%	...
4	0.010%	5.07	0.00175	0.00175	143%	...
5	0.005%	5.31	0.00225	0.00225	111%	...
6	0.0025%	5.35	0.00275	0.00275	91%	...
7	0.00125%	5.36	0.00275	0.00275	91%	...
8	None	5.39	0.0030	0.0030	83%	0.108

The data show that with a p_H of 5.07, Sample No. 4 assays 0.00175. The original assay being 0.0018, there was no deterioration when kept in a temperature of 50° C. for seven months.

According to the data this method of assaying Veratrum preparations suggests that a distilled water dilution gives more consistent readings than does an alcoholic dilution. The alcoholic dilutions show an increase in toxicity in the one-half-hour period and a still greater increase in toxicity after the twelve-hour period, whereas the distilled water dilution gave the same toxicity in both the one-half-hour and twelve-hour periods. The alcoholic dilutions, after the twelve-hour period or readings, show a greater toxicity of the preparation than does the original assay. It is recommended, therefore, that Veratrum preparations be assayed by the Lethal Mouse Method which consists of an intraperitoneal injection of veratrum per Gm. wt. of mouse, 0.00025 Gm. per Gm. of mouse for the drug, and fluidextract and 0.0025 Gm. per Gm. of mouse for the Tincture, dilutions to be made with distilled water and one-half-hour readings observed as the lethal period. The hydrogen-ion concentration factor or p_H values of 5.00 to 4.65 in the fluidextract and tincture of veratrum seem to stabilize its active principles. This is observed more accurately in the heated samples (*Tables II and IV*) and (*Charts II and IV*). The heated samples show the value of the hydrogen-ion concentration factor in stabilizing veratrum preparations. The tincture (*Table IV and Chart IV*) has a greater p_H range and shows that Sample No. 4, with a p_H of 5.07 seems to be about equal to the original assay. The stability of veratrum preparations, rapidly decreases with lessening values of p_H. The active principles of aconite, nux vomica and gelsemium are not injured by increasing amounts of acid. Considering the similarity in pharmacological properties of veratrum and aconite, it is interesting to note that veratrum is easily injured by excessive amounts of acid, whereas aconite requires acid to prevent hydrolysis or deterioration. The above data seem to show that veratrum preparations (the fluidextracts and tinctures) require a p_H of 4 to 5 to prevent deterioration or to stabilize their active principles.

IV. CONCLUSIONS.

1. Fluidextracts and tinctures of veratrum should be assayed biologically; the chemical method is unreliable.
2. Distilled water dilutions are more reliable than are the alcoholic dilutions.
3. It is evident that the hydrogen-ion concentration factor or p_H factor controls the active principles of veratrum.
4. A p_H value of 4 to 5 seems to stabilize these active principles.

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