considered a characteristic crystalline precipitate. In dilutions of 1–1000 or 1–500, needle-like crystals are formed which have a marked tendency to branch, giving the appearance of bushes, Fig. 3. In dilutions of 1–100 the tendency is for the crystals to be arranged in the form of rosettes.

In all the above tests it is well to emphasize that the formation of crystals occurs slowly.

In view of the fact that ephedrine bears a close relationship in chemical structure to both epinephrine and tyramine, the same tests were made with these substances.

In some instances, especially with Kraut's reagent, there is a similarity in the crystals formed which might be confusing. Any doubt, however, may be overcome by the use of ferric chloride solution which gives a color reaction with epinephrine and tyramine owing to the presence of the OH group on the benzol ring. This is not the case with ephedrine.

Caffeine also gives a somewhat similar precipitate with gold chloride and Kraut's reagent, but the absence of a precipitate with platinic chloride and Millon's reagent, as well as many of the other alkaloidal reagents, makes it easy to rule it out.

In order to determine whether the ephedrine could be isolated and detected when mixed with animal tissue, the following experiment was made.

A small amount of the alkaloid was thoroughly mixed with a liberal amount of ground meat and the alkaloid extracted by the Stas-Otto method. The alkaloid was recovered in a very pure form and gave the same microchemic tests as the original drug.

This experiment would give no indication as to how long the drug might remain in the body before being broken down, but it serves to show that it may be recovered and identified if present.

EDITORS NOTE: "A Study of Ephedra Nevadensis," by Ralph E. Terry will follow in the May issue of the JOURNAL.

THE STANDARDIZATION AND STABILIZATION OF ACONITE PREPARATIONS.—PAPER III.

BY EDWARD E. SWANSON AND CHESTER C. HARGREAVES.

1. Review of previous data.
2. Methods of assay.
3. The assay of five series of tinctures and fluidextracts.
4. The hydrogen-ion concentration or p_H value.
5. Conclusions.

In two previous articles (1, 2) by comparing the chemical method U. S. P. IX with the biochemical method now official in U. S. P. X, on a number of aconite drugs, tinctures and fluidextracts, it was found that the chemical method is unreliable. This method assays the total ether-soluble alkaloids, which are similar in chemical properties toward solvents and precipitants, but not similar in toxicity and pharmacological action. The biochemical method, which has been found to be more accurate, determines the total amount of toxic alkaloids in terms of a standard aconitine, which is regarded pharmacologically and therapeutically

as the important alkaloid. It was also found that the tinctures and fluidextracts of Aconite U. S. P. deteriorate rapidly within one year, and furthermore that this loss in activity can be partially or totally prevented by the addition of acetic or hydrochloric acid in the finished percolate or menstruum. The presence of an acid will prevent rapid deterioration and stabilize the activity of aconite preparations for at least three years. The deterioration may be due to the decomposition or hydrolysis of the alkaloids. It was found that by preparing solutions of the pure alkaloids, aconitine, diacetyl aconitine, benzylaconine and aconine in 70% alcohol and 70% alcohol plus acid, the deterioration and stabilization were similar to that of the tinctures and fluidextracts of aconite. Therefore, the deterioration, decomposition or hydrolysis of tinctures and fluidextracts of aconite and of the pure alkaloids,which are prevented by the presence of acetic acid, or hydrochloric acid, may be a question of hydrogen-ion concentration. The hydrogen-ion concentration of each tincture and fluidextract was determined. The preparations containing an acid gave a distinctly higher p_H value, however both acetic acid and hydrochloric acid 36% were used. In determining the hydrogen-ion concentration, hydrochloric acid always gives a more distinct p_H value than acetic acid, therefore the following data represents the use of hydrochloric acid given in terms of absolute hydrochloric acid.

The object of this article is to study more carefully the question of the hydrogen-ion concentration factor, or to determine if a definite p_H value will control the deterioration and stabilization of aconite preparations.

METHODS OF ASSAY.

The efficiency of the biochemical method not only depends upon the aconitine *value*, but also upon a standard method of *technic*. The accuracy of the method depends upon a standard weight of guinea-pigs, the seasonal variation of guinea-pigs, which is controlled by a standard aconitine, the acclimation of guinea-pigs to laboratory surroundings, the use of starved or non-starved guinea-pigs, the dilution of the preparations to be tested, and finally the amount to be injected.

The following method of assay has been used in this work:

Guinea-pigs weighing 275 Gm. to 325 Gm. are starved for 24 hours (by starving the results are far more consistent) they are kept in the laboratory or animal department for one month on a standardized diet. They are usually smaller than the required weight, and are kept until they reach the desired weight, at the same time acclimating themselves to the laboratory conditions. The dose is calculated according to weight. The fluidextract is diluted (1–10) with distilled water; the tincture not requiring dilution. Each calculated dose, diluted with normal saline solution to a total of 1 cc., is injected into the subcutaneous tissue of the abdomen. The lethal dose is determined as the smallest amount, that will kill within 6 hours.

Rowe (3) reported the use of mice, comparing them with the guinea-pig. He found that it required about 6.25 times as much of the drug to give the same results in mice as in guinea-pigs. Considering the difference in cost which is an important factor in all manufacturing procedures, the use of mice should be considered; therefore, the writers have used Rowe's White Mice Method in comparison with the guinea-pig method.

The following series of tests represents the use of more than 1000 guinea-pigs and at least 900 mice.

SERIES I.

Prepared on 2-10-25 a regular lot of fluidextract of Aconite U. S. P. which assayed by the chemical method 0.3785 Gm. of ether-soluble alkaloids per 100 cc. and assayed by the biochemical method 0.000040 Gm. per Gm. weight of guinea-pig. The alcohol content was 67.42%. This lot was divided into six parts, hydrochloric acid added to each part as given in the following table. Each part or sample was assayed chemically and biochemically and the hydrogen-ion concentration determined by the electrometric method. The following table represents one year and 7 months' aging.

Sample number.	HCl Gm. in 100 cc.	p_H 2-10-25.	p_H 3-29-26.	M. L. D. G. 9-20-26.	M. L. D. M. 9-20-26.	Per cent activity pig.	Per cent activity mouse.	Ratio M. L. D.'s.
1	0.072	4.10	4.02	0.000040	0.00040	100	100	1-10
2	0.054	4.40	4.40	0.000045	0.00045	89	89	1-10
3	0.036	4.70	4.90	0.000055	0.00060	72.7	66⅔	1-10.9
4	0.027	4.90	5.00	0.000080	0.00070	50	57	1- 8.7
5	0.009	5.20	5.20	0.000150	0.00090	26.6	44.4	1- 6
6	0.0045	5.35	5.27	0.000150	0.00090	26.6	44.4	1- 6

The deterioration is dependent on the hydrogen-ion concentration and the deterioration increases as the hydrogen-ion concentration decreases. This series of experiments required 0.072 Gm. HCl per 100 cc. or a hydrogen-ion concentration of 4.02 to 4.10 to prevent deterioration.

SERIES II.

Prepared on 2-29-25 a regular lot of fluidextract of Aconite U. S. P., which assayed 0.21 per 100 cc. of ether-soluble alkaloids by the chemical method, and assayed 0.000040 Gm. per Gm. weight of guinea-pig by the biochemical method. The alcohol content was 64.28%. This lot was divided into 6 parts, hydrochloric acid added to each part as given in the following table. The hydrogen-ion concentration was determined by the electrometric method. Each sample or part was tested about one year and 8 months later.

Sample.	HCl Gm. per 100 cc.	p_H 4-29-25.	p_H 1-6-26.	Bio-assay M. L. D. G. 10-16-26.	Bio-assay M. L. D. M. 10-16-26.	Per cent activity pig.	Per cent activity mice.	Ratio M. L. D.'s
1	0.144	3.38	3.56	0.000040	0.00040	100	100	1-10
2	0.072	4.44	4.70	0.000045	0.00045	89	89	1-10
3	0.036	5.20	5.27	0.000060	0.00065	66⅔	61.5	1-10.8
4	0.018	5.45	5.43	0.00015	0.0010	26.6	40	1- 6.6
5	0.009	5.55	5.53	0.00025	0.00175	16	22.2	1- 7
6	None	5.70	5.60	0.00030	0.0025	13.3	16	1- 8.3

The above data show that the deterioration is dependent on the hydrogen-ion concentration, and the deterioration increases as the hydrogen-ion concentration decreases. This series of experiments required 0.144 Gm. HCl per 100 cc. or a hydrogen-ion concentration of 3.38 to 3.56 to prevent deterioration.

SERIES III.

Prepared on 2-29-25 a regular lot of tincture of Aconite U. S. P., which assayed 0.0220 Gm. of ether-soluble alkaloids by the chemical method, and assayed 0.00040 Gm. per Gm. weight

Sample.	HCl Gm. per 100 cc.	p_H 4-29-25.	p_H 1-6-26.	Bio-assay M. L. D. G. 10-30-26.	Bio-assay M. L. D. M. 10-30-26.	Per cent activity pig.	Per cent activity mice.	Ratio M. L. D.'s.
1	0.144	1.58	1.63	0.00040	0.0040	100	100	1-10
2	0.072	2.00	2.10	0.00040	0.0040	100	100	1-10
3	0.036	2.40	2.50	0.00040	0.0040	100	100	1-10
4	0.018	3.20	3.35	0.00040	0.0040	100	100	1-10
5	0.009	4.58	5.05	0.00055	0.0050	72.7	80	1- 9
6	0.0045	4.88	5.53	0.0025	0.0090	16	44.4	1- 3.6
7	None	5.13	6.05	0.0050	0.0175	8	22.2	1- 3.5

The above data show that the deterioration and stabilization is dependent on the hydrogen-ion concentration or p_H value.

of guinea-pig by the biochemical method. The alcohol content was 69.42%. This lot was divided into seven parts, hydrochloric acid added to each part as given in the following table. The hydrogen-ion concentration was determined by the electrometric method. The following table represents about one year and 8 months' aging.

SERIES IV.

Prepared on 3-15-26 a regular lot of fluidextract of Aconite U. S. P., which assayed 0.28 Gm. of ether-soluble alkaloids per 100 cc. by the chemical method, and assayed 0.000030 Gm. per Gm. weight of guinea-pig by the biochemical method. The alcohol content was 64.84%. This lot was divided into seven parts, hydrochloric acid added to each part as given in the following tables. The hydrogen-ion concentration was determined by electrometric method. The following table represents about 9 months' aging.

Sample.	HCl Gm. in 100 cc.	p_H 3-29-26.	Bio-assay M. L. D. G. 12-10-26.	Bio-assay M. L. D. M. 12-10-26.	Per cent activity pigs.	Per cent activity mice.	Ratio M. L. D's.
1	0.36	1.05	0.000030	0.00030	100	100	1-10
2	0.288	1.30	0.000030	0.00030	100	100	1-10
3	0.144	2.00	0.000030	0.00030	100	100	1-10
4	0.072	3.60	0.000030	0.00030	100	100	1-10
5	0.036	4.20	0.000030	0.00030	100	100	1-10
6	0.018	4.60	0.000045	0.00040	66⅔	75	1- 8.8
7	None	5.10	0.000065	0.00060	46.1	50	1- 9.2

The above data show that deterioration is dependent on the hydrogen-ion concentration, and the deterioration begins when the hydrogen-ion concentration is 4.60 or it required 0.072 Gm. HCl per 100 cc. or a hydrogen-ion concentration of 3.60 to prevent deterioration.

SERIES V.

Prepared on 3-15-26 a tincture by diluting fluidextract of Aconite Series IV with 70% alcohol. This tincture assayed 0.028 Gm. of ether-soluble alkaloids per 100 cc. by the chemical method, and assayed 0.00030 Gm. per Gm. weight of guinea-pig by the biochemical method. The alcohol content was 68.5%. The lot was divided into seven parts, hydrochloric acid added to each part as given in the following table, the hydrogen-ion concentration determined by the electrometric method. The following table represents about 9 months' aging.

Sample.	HCl Gm. in 100 cc.	p_H 3-29-26.	Bio-assay M. L. D. G. 12-20-26.	Bio-assay M. L. D. M. 12-20-26.	Per cent activity pigs.	Per cent activity mice.	Ratio M. L. D.'s.
1	0.036	2.23	0.00030	0.0030	100	100	1-10
2	0.028	2.63	0.00030	0.0030	100	100	1-10
3	0.014	3.25	0.00030	0.0030	100	100	1-10
4	0.007	4.52	0.00035	0.0033	85.7	90	1- 9.5
5	0.0035	5.12	0.00060	0.0055	50	54.5	1- 9
6	0.00175	5.37	0.00125	0.0070	24	42.8	1- 5.6
7	None	6.00	0.0035	0.010	8.57	30	1- 2.9

The above data show that deterioration and stabilization is dependent on the hydrogen-ion concentration.

THE HYDROGEN-ION CONCENTRATION OF p_H VALUE.

The series of five experiments seem to show that the hydrogen-ion concentration of p_H value controls value the deterioration and stabilization of aconite preparations. Series I required 0.072 Gm. per 100 cc. or a p_H value of 4.02 to 4.10 to prevent deterioration; Series II required 0.144 Gm. per 100 cc. or p_H value of 3.38 to 3.56; Series III required 0.018 Gm. HCl per 100 cc. or p_H value of 3.20 to 3.25; Series IV required 0.036 Gm. HCl per 100 Gm. or p_H value of 3.60 and Series V required 0.014 Gm. HCl per 100 cc. or p_H value of 3.25. Of course, Series I, II, IV are fluidextracts and should require more acid than the tincture,

but for each series, regardless of fluidextract or tincture, it required a different amount of acid to give the p_H value desired to stabilize the activity, in other words a given constant amount of hydrochloric acid will not control the hydrogen-

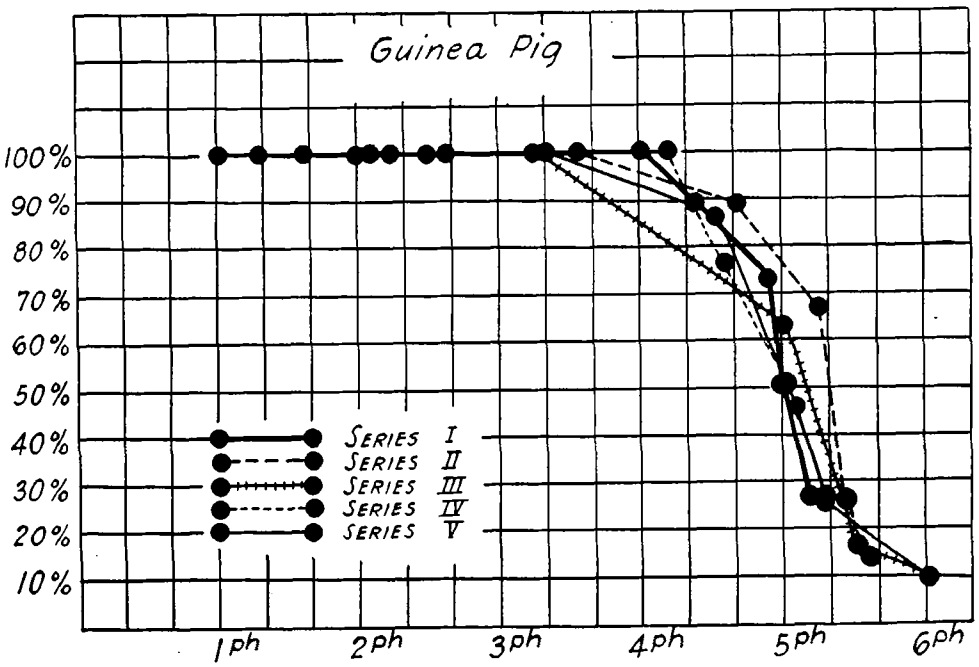

Fig. 1.

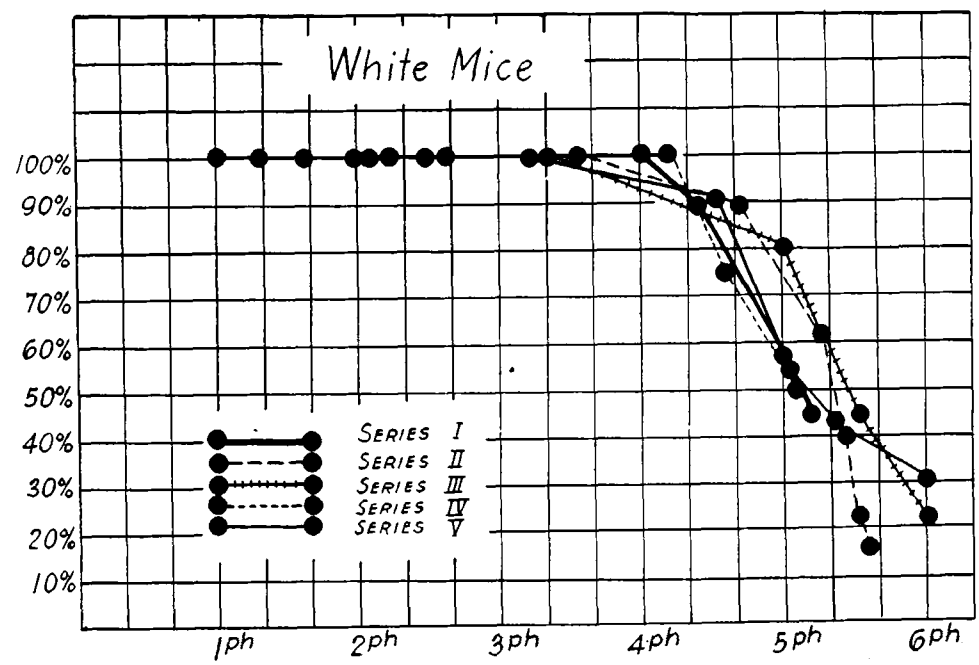

Fig. 2.

ion concentrations of p_H value. The desired p_H value is controlled by the amount of alkaloids and inert substance in the drug. The amount of alkaloids and inert extracts is not constant in all aconite drugs and preparations, therefore requiring different amounts of acid to give the desired p_H value. It seems to the writers that all aconite preparations should have a hydrogen-ion concentration or p_H value of not less than 3.00 or perhaps 2.50 would be a safer figure to stabilize and prevent deterioration.

Figure 1 represents curves of the five series of experiments as determined by the guinea-pig method.

Figure 2 represents curves of the five series of experiments as determined by the mouse method. The two methods agree remarkably well as regard the stability point, but as the deterioration increased they became less correlative. The guinea-pig method indicated a greater deterioration than the mouse method as the p_H value decreased. However, this may be an alcoholic factor. The writers having experimented on both methods found that 10% to 20% alcohol, 95% added to each calculated dose for guinea-pig and mouse, will not change the correlation but that 30% to 40% of alcohol, 95% added to each dose for guinea-pig and mouse, will inverse the correlation or decrease the toxicity on guinea-pigs and increase the toxicity on mice. Thus, in the preparations that have deteriorated the dose will be large for both guinea-pigs and mice, and the greater the deterioration the larger the amount of drug to be injected, and the larger the amount of alcohol that is injected. Weight for weight the mouse required 6 to 10 times as much of the drug as the guinea-pig, and therefore, the mouse receives 6 to 10 times as much alcohol. Furthermore, the drug is given subcutaneously to guinea-pigs and intraperitoneally to mice, which undoubtedly will show some difference. This problem will be more carefully studied and reported later.

CONCLUSIONS.

1. It has been shown that the hydrogen-ion concentration or the p_H value controls the deterioration and stabilization of aconite preparations.

2. It is recommended that tinctures and fluidextracts of Aconite U. S. P. have a p_H value of 2.5 and not less than 3.00 in order to prevent deterioration.

3. It has been shown that the amount of acid required to produce the desired p_H depends upon the amount of alkaloids and inert material present in each lot of drug.

4. The guinea-pig method and white mice method agree remarkably well on standard aconite preparations, but do not agree when the deterioration factor is determined.

The writers are much indebted to Mr. T. Hoover and Mr. A. L. Caldwell for the electrometric determinations and to Mr. H. W. Rhodehamel and Miss L. Carter for suggestions and criticisms.

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