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STUDIES IN THE EXTRACTION AND HYDROGEN-ION CONCENTRATION OF TINCTURE OF DIGITALIS.

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INTRODUCTION.

In a previous communication to THIS JOURNAL the authors (1) studied the relationship between the potency and the hydrogen-ion concentration of tincture of digitalis. In this investigation it was shown that the tincture prepared with dehydrated alcohol as a menstruum, differed from that tincture prepared with 80 per cent in at least three respects. *First*, the tincture prepared with dehydrated alcohol showed a greater hydrogen-ion concentration, *second*, a comparatively negligible amount of ash was present and *third*, the heart tonic value was markedly less than that exhibited by the tincture prepared from the same drug with 80 per cent alcohol.

Since the publication of this work, the comprehensive investigations of Hoekstra (2) have come to the authors' attention. Among other interesting observations, this investigator showed that a tincture prepared with 50 per cent alcohol contained 3.8 cat units of digitoxin, 2.5 cat units of gitalin and 7.3 cat units of bigitalin. Whereas a tincture prepared from the same powder using dehydrated alcohol as a menstruum contained $^3/_8$ the digitoxin content, $^5/_8$ the total bigitalin content and only a trace of gitalin. Wokes (3), after a study of the potency and total solids of tincture of digitalis, concluded that no definite relationship could be established between these factors. Of striking significance is the observation of Stasiak and Zboray (4) who found less residues from the tinctures made with dehydrated alcohol than those prepared with diluted alcohol. In addition, the tincture prepared with dehydrated alcohol showed the lowest potency when assayed by the cat method, but by the frog method the tinctures prepared with dehydrated 70 per cent and 50 per cent alcohol, showed essentially the same potency.

In this study the authors have extended their investigations mentioned in a foregoing paragraph and attempted to correlate their findings with the work of other workers.

EXPERIMENTAL.

Through the courtesy of the Upsher Smith Company and Penick Company, six authenticated samples of digitalis (*purpurea*) were obtained. From these, six tinctures were prepared by the official method. The removal of fat was omitted. Six tinctures were prepared using dehydrated alcohol as a menstruum and from the marcs obtained in this extraction six other tinctures were prepared using 80 per cent alcohol as a menstruum.

The hydrogen-ion concentration of these tinctures was measured by the hydrogen electrode Wilson (5) type. The results were reproducible within 0.1 unit p_H. The ash and acid-insoluble ash of the drugs were determined as well as the ash of the tinctures. The tinctures were assayed for digitoxin by a slight modification of the Keller-Fromme (6) method. In addition the preparations were assayed colorimetrically by the Knudson-Dresbach (7) method using a ouabain standard, and also by the U. S. P. one-hour frog method.

Very recently Dyer (8) critically studied the Knudson-Dresbach method and concluded that the results obtained did not parallel those obtained by the frog method.

The results are shown in Tables I, II and III.

TABLE I.—THE ASH OF DIGITALIS AND ITS TINCTURE.

No.	Drug Ash Per Cent.	Drug Ash Insoluble in HCl Per Cent.	Drug Ash Sol. in HCl Per Cent.	Ash in Tinct. Mg. per L. U. S. P.	Ash in Tinct. Mg. per L. Dehydrated Alcohol.	Ash in Tinct. Mg. per L. Re-extraction.
1	12.75	5.35	7.40	2850	380	1910
2	7.34	0.16	7.18	2430	280	1450
3	7.76	0.06	7.70	2300	280	1310
4	8.59	0.80	7.79	2720	360	1570
5	7.40	0.11	7.29	2160	260	980
6	9.78	1.88	7.90	2760	400	1470

TABLE II.—THE HYDROGEN-ION CONCENTRATION OF TINCTURE OF DIGITALIS.

No.	U. S. P. Tincture, p_H.	Dehydrated Alcohol Tincture, p_H.	Re-extraction Tincture, p_H.
1	5.78	4.10	5.79
2	6.12	4.13	5.91
3	6.25	4.13	6.12
4	6.15	4.12	6.06
5	6.27	4.60	6.15
6	6.08	4.45	5.85

TABLE III.—ASSAYS OF TINCTURE OF DIGITALIS.[1]

		U. S. P. Method.	Knudson-Dresbach.	Keller-Fromme
1	U. S. P. Tincture	100	120	130
2	Dehydrated Alcohol Tincture	84	76	70
3	Re-extraction Tincture	30	100	70

[1] The assay-values are expressed in terms of per cent of potency compared with the U. S. P. standard. The Keller-Fromme standard of 30 mg. per 100 cc. was considered 100 per cent. For assay purposes all six tinctures extracted in the same manner were combined.

DISCUSSION OF RESULTS.

Hydrogen-Ion Concentration.—The results of these investigations on six additional samples of tincture of digitalis confirm the findings of the authors in their previous communication (1). Thus the average p_H of the U. S. P. tincture was found to be 6.11, the p_H of the tincture prepared with dehydrated alcohol 4.25 and the p_H of the tincture prepared by re-extraction 5.98. As it was suggested, this phenomenon is associated with the inorganic constituents of the leaf combined with the organic acids which were studied by Fourton (9). The analysis of the ash showed approximately 76 per cent of potassium carbonate. The U. S. P. tincture

contained approximately ten times more inorganic constituents than the tincture prepared with dehydrated alcohol. With the acids and inorganic constituents present, the U. S. P. tincture exhibits a high buffer capacity as shown by J. K. (10), whereas the tincture prepared with absolute alcohol is practically devoid of inorganic constituents. This is evinced by its low ash content, high hydrogen-ion concentration and low buffer capacity (11).

Nyiri and DuBois (12) showed that the nutrient fluid of the heart can be changed in hydrogen-ion concentration between p_H 5.2 and 7.6 without visible damage to the heart action. These investigators claim full digitalis action to take place only within this range of p_H. Accordingly hydrochloric acid was added to the U. S. P. tincture (1 cc. 0.1 *N* to 20 cc.) to change its p_H to approximately that of the tincture prepared with dehydrated alcohol. The p_H was determined and after six months' standing the biological potency was determined by the U S. P. method. The results are shown in Table IV.

TABLE IV.—POTENCY OF ACIDIFIED TINCTURE OF DIGITALIS.

No.	p_H.	Potency Frog Method—Per Cent.
1	3.83	63
2	4.12	53
3	4.13	79
4	3.94	47
5	3.93	58
6	4.09	58

The average potency of the acidified tincture is approximately 60 per cent of that of the unacidified preparation. The authors are not at present in a position to attribute this loss in heart tonic value to a change in p_H *per se* or the influence which the more acidic preparation might exert on the nutrient fluid of the heart. The indications are that the former condition obtains, as it is well known that upon long standing tinctures of digitalis decrease in potency and increase in hydrogen-ion concentration simultaneously (13), (14), (15), (16).

Ash Content.—The ash content soluble in hydrochloric acid of the six samples of drug was quite uniform, although the total ash content varies from 7.34 to 12.75 per cent. It is interesting to note that Specimen "1" with 12.75 per cent is Minnesota grown digitalis and Specimens 2, 3, 4 and 5 are Canada grown digitalis. The source of Specimen 6 is unknown. Newcomb and Haynes (17) found eleven samples of digitalis gathered personally to vary in ash content from 6.6 to 14.4 per cent.

The influence of the potassium ion in the ash as a factor affecting biologic potency was studied. Solutions of potassium chloride 2500 mg. per liter had no effect on the frog's heart when injected in quantities up to 24 cc. per kilogram. Large doses of a concentrated solution of potassium chloride caused heart stoppage in systole similar to oubain and digitalis. To produce this 2500 mg. per kilogram of a 10 per cent solution of potassium chloride were necessary.

From this it seems evident that the potassium ion as such is not directly responsible for the cardiac response in the re-extraction tincture which contains a relatively large amount of ash. Further, it does not seem to indicate that the deficiency of heart tonic value of the tincture prepared with dehydrated alcohol is due to the small quantity of ash present.

Biological Potency.—Table III shows a comparison of the composite tinctures assayed by three methods. The U. S. P. tincture contained 39.1 mg. of digitoxin per 100 cc., the tincture prepared with dehydrated alcohol 21.0 mg. per 100 cc. and the re-extraction tincture 20.9 mg. per 100 cc. The incapacity of dehydrated alcohol to extract this glucoside by the ordinary process of percolation is indicated by these results and substantiated by the U. S. P. and Knudson and Dresbach methods. The wide discrepancies in the results obtained by the three methods on the re-extraction tincture are exceedingly interesting. In the experiments reported previously by the authors, the re-extraction tinctures showed an average potency of 58 per cent of that by the U. S. P. method. Yet by the Knudson-Dresbach, seven assays of the composite sample showed a potency of 83 per cent of that of the U. S. P. tincture assayed by this method.

The digitoxin extraction (Keller-Fromme) method and the U. S. P. method indicate that the heart tonic value of the U. S. P. tincture is distributed, within the limits of the error of the experiment, between the dehydrated alcohol tincture and the re-extraction tincture. However, the ratio of distribution is vastly different as indicated by the two methods.

Further experimental work will be necessary to determine the rôle of these inorganic constituents found in the U. S. P. tincture, re-extraction tincture and absent in the dehydrated alcohol tincture and pharmacologic activity of the digitalis glucosides in these preparations. In view of the fact that the acidifying of the U. S. P. tincture with hydrochloric reduced materially its potency (Table IV) seems to indicate that the acid-base equilibrium of the tincture is a criterion of potency as measured by the U. S. P. method.

SUMMARY.

1. A greater degree of hydrogen-ion concentration in tinctures of digitalis prepared with dehydrated alcohol than those prepared with 80 per cent alcohol has been established.

2. The ash content of the tinctures prepared with dehydrated alcohol has been shown to be negligible compared with that of the tincture extracted with 80 per cent alcohol.

3. Work of other investigators has been substantiated indicating the inefficiency of dehydrated alcohol as a solvent for digitalis.

4. No conclusions can be drawn as yet regarding the pharmacological rôle of the inorganic constituents of the leaf present in the tincture.

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ALCOHOL-SOLUBLE EXTRACTIVE OF BENZOIN, MYRRH AND ASAFŒTIDA.

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There has been considerable complaint regarding the present U. S. P. method for determining the alcohol-soluble extractive of some drugs because of the loss of volatile constituents during the drying of the extract. This criticism applies particularly to Benzoin, myrrh and asafœtida. Of these, the one that apparently has been causing the most trouble is benzoin. It is suggested by Bickford and Bennett that the alcoholic extractive, including the water, be obtained by means of a Soxhlet extraction apparatus and that the water content be determined by the Xylol method. Other modifications of the U. S. P. method have been proposed but while they appear to give results which probably indicate a truer figure than the U. S. P. method, they are long and tedious and subject to other criticisms as well.

In this investigation there are, therefore, three questions involved, namely: *First*, is the proposed new method (Soxhlet and Xylol) for the determination of alcohol extractive reasonably speedy and satisfactory in that it gives concordant results? *Second*, is the proposed method suitable for other U. S. P. drugs such as, asafœtida and myrrh? *Third*, are these drugs as they occur in commerce at present, meeting the alcohol-soluble extractive requirements of the U. S. P.?

The method (No. 9) proposed by Bickford and Bennett is as follows:

"Weigh 2 Gm. of the sample into a dried and tared paper extraction thimble, using a glass stopper weighing bottle as a container. Extract in a continuous extraction apparatus with 95% alcohol containing about 0.5 Gm. NaOH for 5 hours. Dry and weigh thimble and calculate alcohol extractive matter plus water by difference. Deduct water as determined by xylol distillation method from the result and report as alcohol extract."

Below is a report of a brief study of this method as compared to the U. S. P. method.

Preparation of Samples.—Samples of Siam benzoin, sumatra benzoin and myrrh, consisting of about one pound each, were ground in a mortar and quartered. One quarter was further reduced to a No. 20 powder and used for analysis. In the case of asafœtida about one pound was broken up into small particles and quartered. One quarter was then used for analysis.

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