THE RELATIONSHIP OF POTENCY AND HYDROGEN-ION CONCENTRATION IN TINCTURE OF DIGITALIS.*

A Preliminary Study.

BY JOHN C. KRANTZ, JR., AND JAMES C. MUNCH.

INTRODUCTION.

The relationship between hydrogen-ion concentration and the potency and stability of extractive preparations has warranted the attention of the physical chemist and pharmacologist over the period of the last decade. Accordingly, attention has been drawn to Digitalis preparations. Tainter (1) showed that the physiological activity of freshly prepared infusion of digitalis is independent of its hydrogen-ion concentration. He observed also that the infusions tended to become acid upon standing.

This investigator determined the acidity of the tincture to be the equivalent of an $N/10{,}000$ hydrochloric acid solution (p_H approximately 4.6). Takahashi (2), contrary to the results of Tainter, showed that the addition of 0.05 per cent to 0.1 per cent of hydrochloric acid to infusion of digitalis increased the stability as shown by the frog method of assay.

Smith (3), in his studies of the determination of hydrogen-ion concentration in alcoholic solution, found tinctures of digitalis to range in hydrogen-ion concentration from p_H 5.2 to 5.77. Joachimaglu and Bose (4) showed the stability of tincture of digitalis to be increased by the addition of tartaric acid. These investigators found the p_H of tincture of digitalis to be 5.88, with 0.1 per cent tartaric acid 5.44, and with 0.2 per cent tartaric acid 5.13. In a previous communication to THIS JOURNAL one of us (J. K. (5)) studied the acid-base equilibrium of tincture of digitalis and its buffer capacity. Further the acidity of the infusion (6) and the effect of aging on this preparation was investigated.

Very recently in a survey of pharmaceutical preparations in Virginia, Haag and Jarrett (7) observed that no constant relationship existed between the hydrogen-ion concentration and the heart-tonic value of the tincture. The tinctures studied had a hydrogen-ion concentration generally below p_H 4.50.

With the findings of these investigators in mind the authors began a study of the hydrogen-ion concentration and the potency of the tincture.

EXPERIMENTAL.

The tendency in recent years in the extraction of digitalis for the preparation of the tincture has been to increase the alcoholic content of the menstruum. Thus the German Pharmacopœia has increased the alcoholic strength of the menstruum from fifty per cent to a menstruum of absolute alcohol. This action is supported by the work of Joachimaglu (8), who observed that the total glucosides are extracted from the drug by absolute alcohol.

In the spring of 1930 five samples of digitalis leaves were extracted with 80 per cent alcohol and by the German pharmacopœial method. The hydrogen-ion concentration of these preparations was determined electrometrically using a Wilson (9) type hydrogen electrode.

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The results are recorded in Table I.

TABLE I.—ASSAY OF TINCTURE OF DIGITALIS.

Original p_H—first figures p_H after 16 months—second figures	Sample No. J.	C.	H.	W.	A.
80% Alcohol	5.96	6.02	5.90	6.20	5.75
	5.10	5.43	5.25	5.65	5.73
Absolute alcohol	4.48	4.00	4.30	4.01	3.40
	4.10	4.00	4.25	4.00	3.00
Repercolate, 80% alcohol	5.92	6.10	6.07	6.35	..
	5.20	5.40	5.30	5.90	..
Ash—(Milligrams per Liter).					
80% Alcohol	1890	2050	1320	1360	1960
Absolute alcohol	60	100	20	160	110
Repercolate, 80% alcohol	1450	1130	920	720	..
One-Hour Frog Assay—(Per cent U. S. P. X Standard).					
80% Alcohol	136	100	75	136	75
Absolute alcohol	86	48	60	55	26
Repercolate, 80% alcohol	48	60	48	100	..

Sample J was percolated with 50 per cent alcohol. The p_H of this tincture was 5.96 identical with the value obtained when 80 per cent alcohol was employed. It is apparent from these data that the water removes from the leaves constituents which tend to reduce the hydrogen-ion concentration of the tincture.

Portions of the various tinctures were evaporated to dryness and the residue ashed at a dull red heat. Table I records the data obtained.

The ash content of the tinctures varied tremendously, depending upon the nature of the menstruum employed. With absolute alcohol the ash is negligible—much of the ash not found in the tinctures prepared with absolute alcohol appears in the re-extractions made of the marc with 80 per cent alcohol.

These tinctures were assayed biologically using the One-Hour Frog Method, and the Hatcher Cat Method was employed also upon Sample A.

After a year and four months, the tinctures which were stored in diffused light in partially filled bottles were examined again. The hydrogen-ion concentration was determined—in certain instances the quinhydrone electrode was employed as some of the tinctures had developed the capacity of rapidly poisoning the hydrogen electrode. The results are shown in Table I.

DISCUSSION OF RESULTS.

Hydrogen-Ion Concentration.—The results of these investigations on five samples of digitalis indicate that the tincture prepared with absolute alcohol has a greater hydrogen-ion concentration than that prepared with 80 per cent alcohol. This difference is very marked ranging in the freshly prepared tincture from approximately 1.5 p_H units to 2.3 p_H units. The marc obtained after percolation with absolute alcohol yielded a tincture with 80 per cent alcohol that was practically identical, as far as hydrogen-ion concentration is concerned, with a tincture prepared from the drug with 80 per cent alcohol. This phenomenon is possibly associated with the inorganic salts present in the leaf. The analysis of the ash showed it to consist essentially of potassium carbonate. The acids of the leaf described by Fourton (10) are possibly combined partially with potassium and

extracted by the 80 per cent alcohol yielding a system with a reasonably high buffer capacity (5, 11) having present a strong base combined with a weak acid and also some of the slightly dissociated acid. When, however, absolute alcohol is employed the alkali salts are not dissolved as evinced by the low ash content and the higher hydrogen-ion concentration. This view is substantiated further by the fact that the marc obtained after exhaustion with absolute alcohol yielded to 80 per cent alcohol a tincture of essentially the same hydrogen-ion concentration as the tincture prepared directly from the drug with 80 per cent alcohol. In a subsequent study, the authors anticipate the investigation of the buffer capacity of the tincture prepared with absolute alcohol, which will likely shed more light upon this phenomenon.

Upon standing for more than a year the hydrogen-ion concentration of the tincture prepared with 80 per cent alcohol showed slight increases in most instances. The tinctures prepared with absolute alcohol did not change appreciably in hydrogen-ion concentration.

Biological Potency.—The protocol included shows definitely that there is a vast difference in potency between the tinctures prepared with absolute alcohol and those prepared with eighty per cent alcohol. The question naturally arises as to how to account for this difference in activity which in the instance of sample A was as much as 300 per cent. As mentioned previously (8) the glucosides of the leaves were found by Joachimaglu (8) to be soluble in absolute alcohol. Furthermore, the extraction with absolute alcohol would not preclude the extraction of the saponin gitin, which is claimed by Cloetta (12) to potentiate the activity of the digitalis glucosides. It is possible that the difference in cardiac response could be attributed to the difference in hydrogen-ion concentration *per se*. In this respect Nyiri and DuBois (13) showed that the nutrient fluid of the heart can be changed in hydrogen-ion concentration within a range of p_H 5.2 to 7.6 without visible damage to heart action.

The tinctures prepared with absolute alcohol were considerably below this range. In fact Sample A, which exhibited a p_H of 3.40, was only one third the biological potency of the tincture prepared from the same drug with a p_H of 5.75. These investigators claim full digitalis action to occur within their established p_H 5.2 to 7.6 range. In this connection, it is of interest to note, that tinctures of digitalis upon standing decrease in heart tonic value accompanied simultaneously with an increase in hydrogen-ion concentration. Work along this line has been conducted by Joachimaglu (4), Krantz and Carr (14), Rojahn (15), and Macht and Krantz (16, 17, 18).

Our attention was then drawn next to the mineral constituents of the ash to account for the difference in behavior. During the past two decades much important work has been published concerning the influence of electrolytes on cardiac response. It has been shown (Sakai (19), Daley and Clark (20)) that the "pace maker" may be shifted by changes in the Ca^{++}:K^{+} ratio.

An analysis of the ash obtained from tincture A showed 76 per cent potassium carbonate, 0.3 per cent calcium oxide, 3.0 per cent phosphorous pentoxide and about 1 per cent acid-insoluble material. The ash showed a positive reaction for sodium (flame test) and yielded a precipitate with zinc uranyl acetate (21).

The ash contained 9.7 per cent of chlorine, presumably in the form of sodium and potassium chlorides—corresponding to 15.8 per cent of the former. The ash

did not respond to the bismuthate (22) test for manganese and gave no characteristic reaction with *o.p.* dihydroxy-azo-*p*-nitro-benzene (23), indicating the absence of magnesium. The remainder, approximately 5 per cent of ash, was assumed to be sodium carbonate.

The quantity of calcium present in the ash is insignificant. However, the quantity of potassium present may play a rôle. Fühner (24) has shown that potassium chloride in concentrations of 500 to 1000 mgm. per liter is sufficient to cause stoppage of the perfused frog heart in diastole. The tinctures prepared with 80 per cent alcohol contained about 15 mgm. of ash in 10 cc. or 1500 mgm. per liter, 1000 mgm. of which may be considered as soluble potassium salts. This factor presents itself in no uncertain terms when one considers that the re-extracted marcs, with 80 per cent alcohol from the absolute alcohol tinctures, were characterized by a potency ranging from 48 to 100 per cent.

Thus this investigation has opened three interesting questions which the authors propose to pursue further. One, is it the increased hydrogen-ion concentration in the tincture prepared with absolute alcohol that is responsible for the diminished potency? Two, is it the presence of potassium ions which augments the potency of the tincture prepared with 80 per cent alcohol? Three, if either of these conditions obtain there arises the question of the suitability of intravenous methods as criteria of the therapeutic efficacy of a product intended to be administered orally.

SUMMARY.

1. A study of the potency and hydrogen-ion concentration of tincture of digitalis has been carried out.

2. The influence of alcoholic concentration upon hydrogen-ion concentration and potency has been studied.

3. Certain theoretical aspects of the problem have been discussed.

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DERIVATIVES OF TRIBROM-ETHANOL: (AVERTIN).

A PRELIMINARY REPORT.

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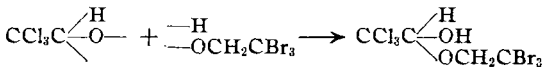
Within the last two or three years, this compound has come into extensive use therapeutically as a local anæsthetic. Its one disadvantage, due to its insolubility in water, is that it must be administered internally in amylene hydrate solution. If this disadvantage can be overcome, the improvement will, no doubt, add materially to the clinical application of this new remedy. Hence, to find a means of introducing avertin into the system in the form of some water-soluble derivative, which at the same time would exert the physiological action of avertin, or which might hydrolyze to liberate avertin itself presents an interesting problem of chemotherapy. However, the writer was not introduced to the study of this compound from this particular point of view.

A systematic study of the readiness with which aliphatic alcohols formed chloral alcoholate addition products with liquid chloral was made in 1930.[1] In connection with the formation of these alcoholates, it seemed of extreme interest to study the possible addition of avertin, which is, structurally, tribrom-ethanol, to chloral, in a manner analogous to that of ethyl alcohol to chloral, namely:



The corresponding bromal compound has also been prepared. Of greater interest, however, are ether-like compounds prepared from tribrom-ethanol. Of these a variety suggested themselves. A preliminary report on some of these is herewith recorded.

EXPERIMENTAL.

Chloral Tribromethylate.—To 1 Gm. of crystalline avertin, a generous quantity of which was supplied by the Winthrop Chemical Research Staff, in 10 cc. of heptane, a mole equivalent of liquid chloral (0.6 Gm.) was added. The reaction mixture was refluxed for half an hour, using an air condenser, allowed to cool, then placed in a refrigerator. Within an hour a crop of glistening white plates was obtained. These were removed by filtration and dried thoroughly on a porous plate. They melted at 68–70°. Recrystallized twice from heptane, the melting point remained constant at 69–70°. This first attempt resulted in a comparatively low yield of 0.92 Gm. or 62 p. c. of the theoretical. Subsequent attempts with 2 and 5 Gm. lots yielded 76 p. c., 83 p. c. and 84 p. c., respectively, of addition compound.

[1] JOUR. A. PH. A., 19 (1930), 320.