SCIENTIFIC SECTION

THE p_H AND POTENCY OF DIGITALIS INFUSIONS.*

BY MAURICE L. TAINTER, M.D.

The object of this paper is a brief summary of observations made during the past three years on the changes in p_H (hydrogen-ion concentration) of digitalis infusions and the bearing of these on their physiological activity. Determinations of the p_H of some tinctures are also included. Except for the work of Joachimoglu and Bose (1) on the tincture, which appeared during the progress of this work, no information along this line has been available. The results that were obtained are interesting because of the unsuspected and rather high acidity of digitalis preparations found without demonstrable relationship to the potency of the infusion, and because they indicate the nature of the decomposition process.

p_H.

The first observations were made on a series of 19 infusions prepared in the students' laboratory from the same lot of leaves with tap water, according to the U. S. P. IX. The median p_H, determined colorimetrically in the usual manner and using the standards of McIlvaine (2), of the fresh infusions was 6.5 (range 6.3 to 6.9). On standing the p_H increased to 6.9 at the end of the fourth day followed by a decrease to 6.2 (greater acidity) at the end of 28 days and then it remained unchanged during the following two months. Essentially the same changes on standing, but a higher degree of acidity, were observed in three other infusions made with distilled water and from two other lots of leaves.[1] The results with these are presented in Fig. 1 (curves of infusions II and III at room temperature) and in Fig. 2 (curve of unpreserved infusion). Their median p_H when fresh was 6.1 and after standing different periods, 5.8. The change toward higher acidity coincided with the appearance of moldy growths. These results suggest that the preparations made with tap water were less acid than those made with distilled water, though molds occurred in both. On the other hand, it may be that the different lots of leaves used in the infusions with distilled water possessed a higher degree of acidity. That digitalis leaves themselves possess a high degree of acidity, is indicated by the following results with tinctures.

A tincture made from leaves used in the students' infusions had a p_H of 4.3, and 10 other tinctures made from 8 different lots of leaves, including 5 commercial tinctures, gave an average p_H of 4.6 (range 4.3 to 4.8). Obviously, the tinctures possessed a degree of acidity nearly equivalent to that of $n/10{,}000$ hydrochloric acid. These results agreed fairly well with those reported by Joachimoglu and Bose (1) who found a p_H of 5.88, and leave no doubt that the true acidity of tinctures of digitalis is rather high. A lower acidity of the infusion might be expected

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[1] These leaves were supplied by Upsher Smith, Inc.

from its lower (about one-sixth) content of leaves. Such was actually found, since the acidity of the infusion was about one one-hundredth, when fresh, up to one-tenth on standing, of that of the tinctures. This lower acidity might be a factor in its poor keeping qualities, so that by raising the acidity the decomposition might be delayed or prevented. The effect of acidity and certain other factors were tested in the following experiments with the infusion.

One infusion was acidified to a p_H of 3.5 by the addition of 0.01 per cent hydrochloric acid, and a second to a p_H of 3.7 by adding 0.1 per cent lactic acid. From the curves in Fig. 1 it is seen that the hydrogen-ion concentration showed only slight fluctuations during the two months' period of observation. However, decomposition occurred as rapidly as in the infusions described above, as indicated by the presence of clouding, surface growth and foul odor at the end of the fourth day. An infusion made alkaline to a p_H of 8.0 by the addition of 1 per cent sodium bicarbonate became less alkaline (p_H 7.3) on standing and also developed a profuse growth and putrid odor. Therefore, increasing or lowering the acidity does not help in preserving the infusion.

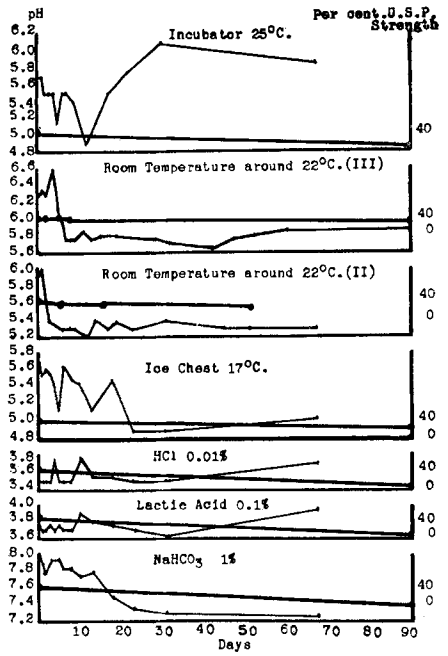


Fig. 1.—Effect of temperature and the addition of acids and bicarbonate on the changes in p_H (hydrogen-ion concentration) and potency of digitalis infusions. The light irregular lines represent p_H changes, and the heavy straight lines, potency.

All the preparations described above had been kept at room temperature which fluctuated around 22° C. Two additional infusions were observed at constant temperatures, one being kept in the incubator at 25° C., and the other in the ice chest at 17° C. The infusion in the incubator (Fig. 1) became less acid on standing, than the infusion in the ice chest although the acidity during the first 12 days was somewhat greater and occurred more rapidly. On the twelfth day it showed cloudiness and surface growth accompanied by a simultaneous increase in p_H, *i. e.*, decrease in acidity. The infusion in the ice chest increased in acidity more slowly, with fluctuations, and required about 15 days longer to reach a constant p_H than was the case with the preparations kept at room temperature. This infusion remained clear and showed no signs of bacterial growth at the end of 6 months' time. From this it appears that a low temperature such as in an ice chest does not prevent, but delays, the natural increase in acidity, and would aid in keeping the preparation pharmaceutically more elegant.

Attempts were next made to prevent the growth of organisms in infusions by sterilization with heat, and by the addition of various preservatives. Seven infusions were made from the same lot of leaves, the results of which are shown in Fig. 2. One of these infusions was sterilized in an Arnold steam sterilizer

for one-half hour on each of 3 successive days. Four were saturated with chloroform, thymol, oil of cloves and oil of cinnamon, and to one was added 10 per cent of alcohol according to the U. S. P. X. The remaining infusion was an unpreserved control prepared according to the U. S. P. IX. During the 2 months' period of observation, the heat sterilized, alcohol, chloroform and cinnamon treated preparations remained sterile as indicated by negative bacteriologic cultures and microscopic examination.[1] There was some clouding of the cinnamon infusion immediately after adding the oil, but no further change in this preparation was observed thereafter. The changes in hydrogen-ion concentration of these four infusions were quite different from those of the other three. There was a rather slow but progressive increase in acidity, which was practically the same in each, *i. e.*, from about p_H 6.1 to 5.7 at the end of 55 days. On the other hand, the remaining three preparations showed sediments, and marked growths on their surfaces, and possessed a pronounced odor on the fourth day. The presence of demonstrable growths coincided with the beginning of the marked p_H changes, *i. e.*, at about p_H 6.1. The unpreserved control showed a pronounced increase in acidity during the first seven days which lasted till the end of 20 days and then was followed by a slow change towards alkalinity, though it still remained quite acid (p_H 5.9) at the end of 57 days. The infusions preserved with thymol and oil of cloves underwent a rapid increase in p_H and became nearly neutral (p_H 6.95 and 6.9) at the end of 57 days. That is, instead of increasing in acidity as was the case with the other infusions, their acidity diminished.

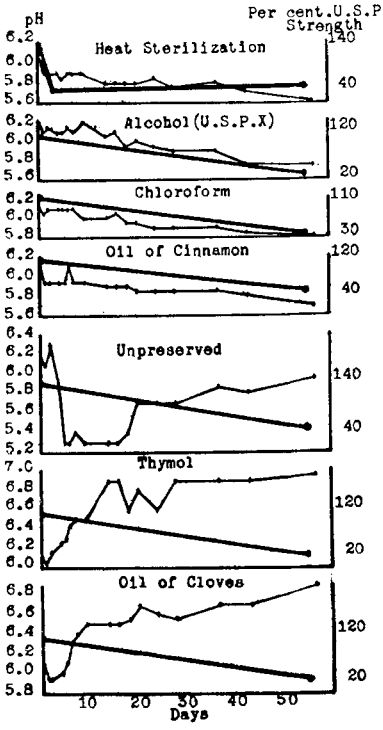


Fig. 2.—Effect of heat sterilization and preservatives on the changes in p_H (hydrogen-ion concentration) and potency of digitalis infusions. The light irregular lines represent p_H changes, and the heavy straight lines, potency.

These results give some idea of the cause of the p_H changes observed. That is, in those infusions showing no growth of organisms, the p_H changed very little, while in those with growth, there were marked changes, the direction of which was determined presumably by the type of organisms predominating. Therefore, the conclusion seems justified, that the marked changes in p_H were conditioned on the presence of contaminating organisms.

POTENCY.

In addition to observations on the p_H, bioassays of the infusions were made from time to time, using the official one-hour frog method, and standardizing the frogs

[1] I am indebted to Dr. E. C. Dickson of the Department of Public Health and Hygiene, who kindly made the bacteriologic cultures.

at each assay against the same specimen of crystalline strophanthin (Merck). The changes in potency of the various preparations are represented by the heavy lines in Figs. 1 and 2 and expressed in per cent of U. S. P. strength. It is seen that the p_H of the fresh infusions bore no relation to their activity. Thus the 2 room-temperature infusions (Fig. 1) had initial p_H values of 6.3 and 5.9, but their activity was identical, *i. e.*, about 40 per cent, while the unpreserved infusion (Fig. 2) prepared from different leaves had a p_H of 6.1, and an activity of 140 per cent. The two infusions kept at room temperature (Fig. 1) and the one in the ice chest lost only about half their activity during the ninety-day period of observation. However, an infusion that was kept warmer, that is, in the incubator at 25° C., showed a complete loss of activity during the same time. The infusions to which small quantities of hydrochloric and lactic acids and bicarbonate were added, were found entirely inactive at the end of this period. Apparently, the increase in acidity accelerated the loss of activity in accordance with the well-known action of acids on glucosides. The destructive effect of bicarbonate was in agreement with the previous observations of Watanabe (3) and others, and contraindicates the use of alkalies, for instance citrate (4), as preservatives for the infusion. These results suggest that the hydrolytic cleavage of the glucosides, which goes on slowly at room temperature and the natural p_H, is accelerated by an increase in acidity or alkalinity as well as by a moderate increase in temperature.

There was no conclusive relationship between loss of activity and contamination with living organisms. That is, the heat-sterilized preparation (Fig. 2) lost the greater part of its activity at the time of sterilization, and thereafter showed no further loss of activity, while the other three sterile infusions (alcohol, chloroform and cinnamon) deteriorated at about the same rate and to the same extent as the three contaminated preparations (unpreserved, thymol and cloves). Since the direction of the p_H changes in these infusions was widely variable, while the rate of deterioration was the same, it is indicated that the loss of potency was not dependent upon, nor reflected in, the spontaneous changes in hydrogen-ion concentration. This result and the fact that deterioration occurred independently of growth of living organisms, definitely relegate the progressive loss of activity of digitalis infusions to hydrolytic cleavage of the glucosides. This deduction is fortified by another fact, namely by the greater stability of alcoholic preparations, for instance, the tincture.

CONCLUSIONS.

1. Infusions of digitalis tend to undergo a spontaneous increase in acidity on standing, whether made with distilled or tap water, or by the methods of the U. S. P. IX or X, and also independently of temperature changes and preservatives.

2. The presence of growing organisms may modify the direction or extent of the p_H changes.

3. The loss of potency, as indicated by the official one-hour-frog method, is not prevented or altered by the addition of such preservatives as alcohol 10 per cent (U. S. P. X), and chloroform, thymol, oil of cloves or oil of cinnamon to saturation.

4. Deterioration is as rapid in sterile as in contaminated infusions, and seems to be due to the hydrolytic cleavage of the glucosides.

5. The physiologic activity of fresh, standing and decomposed infusions is independent of their p_H.

6. The true acidity of tinctures of digitalis is rather high, being nearly equivalent to that of a $n/10{,}000$ hydrochloric acid.

REFERENCES.

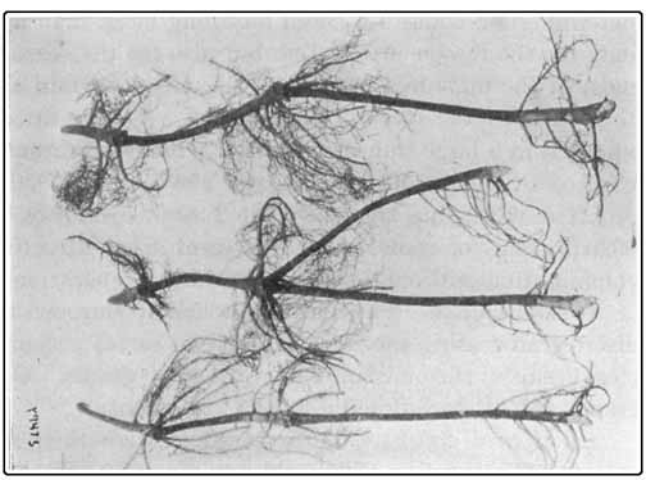
(1) Joachimoglu and Bose, *Arch. Pharm. exp. Path.*, 102, 17 (1924).
(2) McIlvaine, *Jour. Biol. Chem.*, 49, 183 (1921).
(3) Watanabe, *Tohoku, J. Exp. Med.*, 4, 98 (1923).
(4) Plaut Research Laboratory; Lehn and Fink, *Am. Jour. Pharm.*, 97, 456 (1925).

PHYTOCHEMICAL NOTES.*[1]

95. A CHEMICAL STUDY OF THE RHIZOME AND ROOTS OF PODOPHYLLUM PELTATUM L.

BY H. L. KUESTER.

Collection and Drying.—The rhizome and roots used in the experiments subsequently recorded were collected in the vicinity of Madison between the dates of October 1 and November 12, 1924. Part of the drug was harvested in the Pharmaceutical Garden where two rows of the plant have been cultivated under natural shade since 1920 or 1921. Part of the material was from wild plants that had not been transplanted previously. In harvesting the material in the Garden, the buds were separated and replanted. So far as the wild material is concerned, the same course was followed in order to insure against extermination of the plant.



Rhizomes and Roots of Podophyllum Peltatum.

RHIZOMES AND ROOTS OF PODOPHYLLUM PELTATUM.

The rhizomes show the striking difference in growth during the season of 1923 as compared with that during the season of 1924. The bud end of the new growth of rhizome, together with the roots near the bud were cut off for replanting. The remaining portion of the rhizome, together with the roots attached thereto, was dried to drug and used in the investigation here reported.

The soil was carefully removed from rhizome and roots by washing. The imperfectly drained material weighed 18,290 grams. Air dried, it weighed (November 21st) 5950 grams. The loss of 12,340 grams represents 67.4 per cent of the original weight. This is probably somewhat too high because of the imperfect

* From the laboratory of Edward Kremers.

[1] Presented to Scientific Section, A. PH. A., Des Moines meeting, 1925.