the limits of this paper, we have no hesitation in saying that as yet no explanation from advocates of the change have appeared valid to us. The argument most frequently stressed, the argument that the addition of another two grams of citric acid prevents precipitation, is, in our opinion, based upon faulty reasoning. The true solution of the precipitation problem is solved by two factors: (*a*) complete sterilization of the finished solution (including bottles and stoppers) and (*b*) proper sealing of the bottle. We therefore express the hope that the next Revision Committee will direct the lessening of the citric acid content of the solution to 33 Gm. per 350 cc.

CONCLUSIONS.

1. The test for limit of total acid in Solution of Magnesium Citrate has stood the scrutiny of hundreds of analyses and has withstood the fires of the rigidly critical cross examination of litigation.

2. That part of the test relating to "minimum of acidity" is somewhat too severe, is unnecessary and should not be mentioned in the monograph of U. S. P. XI.

3. Statements as to the rapid loss of citric acid by decomposition after the bottle is opened are not borne out by data given in this paper.

4. The pharmacopœial requirement as to ashing *at dull red heat* should be observed. However, heating to white heat for one-half hour does not seriously affect the "assay."

5. The alkalinity of the resultant ash should be determined by residual titration rather than by the direct titration method given on page 431 of U. S. P. X.

6. It is our opinion that the increase of the citric acid content of the 350-cc. bottle of Solution of Magnesium Citrate from the 33 Gm. of U. S. P. IX to the 35 Gm. of U. S. P. X was inadvisable and unnecessary.

ACKNOWLEDGMENTS.

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COLLEGE OF PHARMACY, COLUMBIA UNIVERSITY,
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EXPERIMENTAL INVESTIGATIONS CONCERNING THE STANDARDIZATION AND THE PHARMACOLOGY OF HEART TONICS.*

(With a New Assay Method.)

BY WILLIAM NYIRI, M.D., AND LOUIS DUBOIS.

During the last two years we have carried out research work on heart tonics at the Rutgers College of Pharmacy in Newark, N. J., and we take the liberty to report, briefly, on the results of our investigations, giving you the principal points

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of our findings. The shortness of time prevents us from giving proof of our statements; however, we hope that the discussion will give us the opportunity to go into some details.

Our work may be subdivided into two parts:

I. Standardization of Heart Tonics.

II. Pharmacology of Digitalis, with special reference to the relationships of calcium- and hydrogen-ions to digitalis.

Fifty cats, more than 300 rabbits and nearly 4500 frogs have been used for these experiments.

I.

We studied each important factor of digitalis standardization separately and came to the following conclusions:

A. Warm-blooded animals are to be preferred as test material to animals lower in the animal scale, and to plants. In particular, we studied the variation obtained in the results with the use of the frog as test animal. We successfully carried out the U. S. P. frog assay, the timeless method, the intravenous method, and made experiments with the isolated heart on the cannula by Straub. The individual variation of these experiments was very high and showed the same range even when working on the Straub heart. We conclude from these experiments that this variation is inherent to the frog heart itself.

B. With our present knowledge of the response of living test material to the action of digitalis, the number of animals necessary for a single determination should be left to the judgment of the individual worker and dependent upon his experience. Biometric methods, in general, should be used with the utmost care to answer the above question. The formula of Van Wijngaarden, which at present is generally accepted and endorsed by the Hygiene Committee of the League of Nations, allows too wide a range of variation and does not fulfil the purpose for which it has been recommended.

C. The best way of administering heart tonics in the assay is the intravenous injection.

D. The new intravenous anesthesia is to be preferred over the former methods of narcosis in animal experimentation, in general, for the standardization of heart tonics in particular.

E. The drop of the blood pressure to zero, as shown by the kymographic tracing, approaches closest the theoretically expected end-point of the experiment and thus is to be preferred to the observation of the stoppage of the heart and the general death of the animal.

F. The time of the individual experiment should be kept as uniform as possible. In view of our newer knowledge of the distribution of digitalis in the body and with reference to the differences in the pharmacological action of the various active constituents of the heart tonics, this is one of the criteria of a successful assay. Considerable differences as to beginning and duration of the action of these constituents on the heart make this precaution necessary.

G. One of the main handicaps of the different methods of digitalis standardization in use at present is the fact that both tinctures and infusions need preparation prior to the test. These procedures, as evaporation of alcohol or water and

great dilution of the alcoholic solution throws out active constituents thus damaging the strength of the preparation to be tested. The basic requirement for a practical assay method, the purpose of which is to give information about the quantitative action of digitalis preparations, should avoid any such damaging manipulation.

H. Based on the study of these principal factors, we worked out a practical method of digitalis standardization using the rabbit as test animal. This method has the following advantages: The animal material is always easily available. The end-point of the assay obtained by means of the drop of the blood pressure, supplementing the test by the use of ouabain, is definite and as close to the theoretical end-point as may possibly be expected. Rabbits have a higher resistance to heart tonics than other warm-blooded animals. Tinctures, therefore, need only be diluted one to four, which does not interfere with the test. The method allows the testing of drugs of high concentration as well as drugs of great dilution without preliminary damaging manipulations of the heart tonics.

II.

The results of our experiments concerning the relationships of calcium- and hydrogen-ions to digitalis may be briefly summarized as follows:

A. Full digitalis and strophanthine poisoning of the heart with all eventual toxic stages takes place even in the absence of calcium ions.

B. Increased Ca^{++}-concentration to at least four to five times the normal reinforces and hastens the action of digitalis.

C. The acid-base equilibrium of the nutrient fluid of the heart can be changed within the range of a p_H of 5.2 to 7.6 without visible damage to the heart action. The heart takes up the excess of H^+ or OH^- ions within the above range within a very short time, thus reëstablishing the physiological balance.

D. Full digitalis action occurs with changes of the p_H within the above-mentioned range.

E. Typical digitalis poisoning also takes place when the heart works under the combined influence of calcium and atropine as well as of calcium and adrenaline.

The experiments show that the toxic action of digitalis and strophanthine on the heart is *sui generis* and independent within reasonable limits from changes in the concentration of the surrounding ions. The reinforcing effect of calcium on digitalis encourages their combined therapeutic use.

ABSTRACT OF DISCUSSION.

James C. Munch stated that he was very much interested in this work; that he had been collecting various methods of standardization and for Digitalis he had thirty-eight bio-assay methods. He stated that the method presented had not been previously reported in literature and therefore it may be called a new method, and inquired relative to the lethal dose of ouabain to the rabbit and whether the dose was reasonably consistent throughout the year.

The author replied that the lethal dose is about twice as high to rabbits as to cats; instead of 100 mg. it is around 190 mg. per kilo.

James C. Munch further inquired whether part of the digitalis was first given and then ouabain. The author stated that the test required about 30 or 40 minutes. There was a waiting period of about twenty minutes before administering ouabain.

The results of these investigations, which have been made possible by a research grant of Merck & Co., Inc., Rahway, New Jersey, appear in a series of four publications in the *Journal of Pharmacology and Experimental Therapeutics.*