A NOTE ON THE EPHEDRINE CONTENT OF EPHEDRA VULGARIS VAR. HELVETICA.

BY PETER MASUCCI AND KO SUTO.

Chen[1] has studied the ephedrine content, the alkaloid in *Ephedra vulgaris* var. *helvetica*, and found it to vary from 0.018 to 0.091 per cent. These results were obtained on three samples assayed according to the method given in the U. S. P. IX under Belladonna Root.

Using the same method of asssay recommended by Chen, and the same factors, namely: one cubic centimeter of $N/10$ H_2SO_4 is equal to 0.016513 gram of ephedrine, we obtained a much higher content of ephedrine, from a sample of Ma Huang. The sample was from a small lot of drug shipped directly to us from China and indentified botanically as *Ephedra vulgaris* var. *helvetica*. The ash content was found to be 14.97%. The alkaloidal content was determined by two different analysts, who found 0.305 and 0.298% respectively.

Three fluid extracts were made from this drug using various amounts of alcohol in the extraction fluid. The fluid extracts were assayed using the same method and the alkaloid found was 0.3117; 0.4623 and 0.3060 grams per 100 cc.

Recently we had occasion to assay a sample of *Ephedra vulgaris* submitted by a dealer in crude drugs. This was found to contain 0.515% ephedrine.

The ephedrine content of the two samples of *ephedra vulgaris* assayed by us was so much higher than that reported by Chen that we think this is worth while recording in literature.

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August 17, 1926.

DIGITALIS STANDARDIZATION AT GENEVA.

BY E. A. BILHUBER.

The Health Committee of the League of Nations met early in September, 1925, at Geneva, and adopted reports from a committee on the biological standardization of certain remedies. This included methods for the biological standardization of digitalis and digitalis preparations. The reports by Dr. Gilchrist of Edinburgh, and Dr. Andrus of Baltimore, on the clinical comparison of samples of digitalis and its preparations were discussed and Professor Magnus presented memorandum on biological assay.

Professor Cushny dealt with certain difficulties which he had experienced in the application of a modified Hatcher method on the anesthetised cat, the results seeming to indicate that the cats fell into two classes, one with a definitely higher resistance to digitalis than the other. Cushny also laid stress on the methods of assay in which the frog was used as they may be expected to yield results of a high order of accuracy. Professors Pick, Straub and Rost all reported on the frog method as a valuable means of assay.

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[1] A Pharmacognostic and Chemical Study of Ma Huang (*Ephedra Vulgaris var. Helvetica*) K. K. Chen, JOUR. A. PH. A., Vol. 14, p. 189 (1925).

A sub-committee discussed in detail the various methods for the biological standardization of digitalis including, in addition to the methods using the cat and the frog, the Knaffl-Lenz guinea-pig method; the method proposed by Krogh, using mice; and the method of Mansfeld, using isolated fragments of the *sinus venosus* of the frog's heart. Their resolutions were unanimously accepted by the Conference.

These resolutions provided for the maintenance of a quantity of standardized powdered leaf of the digitalis purpurea. This standard is to be prepared by the mixture of ten different powders, made from leaves properly dried at 55–60° C., and adjusted by biological assays carried out by Professor Magnus. Its activity is to be tested annually and provision made for the upkeep of a full strength (=10%) powder in sufficient quantity. The preparation is to be distributed in sealed brown glass ampuls for international use.

The committee did not recommend any particular method of extraction as the only correct one.

As methods of biological assay the committee recommended as sufficiently accurate, both the frog method and the cat method as modified by Magnus from that of Hatcher. The preparation of the digitalis extract and frog method only will be given in detail as being of greater interest and it is suggested that it be compared with the U. S. P. one-hour frog method of standardization and its forerunner, the original Gottlieb one-half-hour frog method.

The report read:

"A. *Preparation of an extract of digitalis leaves with absolute alcohol.*

"One gram of digitalis leaves, coarsely powdered (B. 20 = mesh of about 0.75 mm.) and dried to constant weight over sulphuric acid, is allowed to stand for 24 hours at room temperature with 25 cc. of absolute alcohol, with occasional shaking in a closed spherical flask of about 100cc. content. The mixture is then boiled for 30 minutes with a reflux condenser, on a sand-bath over the smallest possible flame, and, while still hot, is filtered through a plain filter of about 9cm. diameter. The residue is washed with absolute alcohol on the filter until the filtrate becomes colorless. The combined filtrates are slowly evaporated in a thin-walled, tared watch-glass, on a boiling water-bath to 5 cc. (about 4.5 gr.), the drying of any portion being carefully avoided.

"The concentrated extract, while still hot, is transferred with the aid of distilled water to a graduated flask, and made up to 25 cc. with distilled water. By this procedure one obtains an emulsiform, greenish solution in weak, watery alcohol. This must be used immediately for the test."

B. *Assay of the extract, obtained as described under (A) on frogs, by determination of the minimal lethal dose by the so-called unlimited-time method.*

For the test only healthy male frogs must be used (grass frogs, *rana temporaria* or *rana pipiens*), kept under constant conditions and weighing up to 40 grams each. The body weight of the frogs, kept for several hours in the laboratory in a moist glass case, is determined immediately before the injection to an accuracy of 0.5 gram, after drying the skin and expressing the urine.

"The extract prepared as above described is injected into frogs, through the mouth, into the breast lymph-sac, with a syringe graduated in hundredths of a cc. Larger quantities than 0.3 cc., or with weakly active preparations 0.5 cc., should not be injected into the breast lymph sac; if necessary, the injections are to be made, in such cases, also into one or both of the lymph sacs of the thighs."

The following signs of intoxication appear: Within $^1/_2$-hour to 2 hours after the injection restlessness, air-hunger, formation of froth, paralysis and, in the course of four hours, stoppage of the heart. The criterion for the determination is that the stoppage is either systolic or rapidly transformed into systole.

The orientating tests are carried out as follows: Doses differing by 20 per cent per gram of frog are injected, one or two frogs being used for each dose.

The final determination can be made by the following procedure:

The mean between the smallest active and the greatest inactive dose is the first approximation. By further, more exact determination, with four to six frogs on each dose, the final value can be obtained with an accuracy of 10 per cent. The determination is completed when, of two doses differing by 10 per cent, the higher kills a majority of the frogs injected, the lower a smaller number. The value is expressed as a percentage of the standard preparation, which is tested at the same time and in the same manner. Only such leaves shall be passed for issue as differ from the standard preparation by not more than 25 per cent.

The assay of digitalis tinctures is made in the following manner:

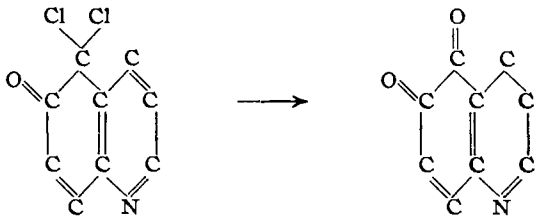
10 cc. of the official tincture (= 1 gram of leaves) are concentrated on the water-bath at temperatures not above 60° C. to 5 cc. volume, washed into a measuring flask with distilled water, and made up to 25 cc. The assay is made according to the same method as described above for digitalis leaves.

THE PREPARATION OF QUINOLINE ORTHO QUINONE AND ITS RELATION TO THE THALLEIOQUIN REACTION.

BY GEORGE W. HARGREAVES.*

In a previous paper (1), a study of the thalleioquin reaction, for the detection of quinine, was made and a new modification discovered which strengthened the view that a quinone is formed in the reaction. In order to shed further light upon the chemistry of this reaction, the preparation of quinoline *o*-quinone was undertaken.

Only two references were found to this body in the literature. The first was by Mathëus (2) who first prepared it, and the second by Fühner (3), who by passing chlorine into a cold solution of the hydrochloride of 6-hydroxy-quinoline obtained a 5,5-dichlor, 6-keto-quinoline, which was colored green or blue by ammonia and led him to believe that the quinoline quinone of Mathëus was formed intermediately:



The method used in the preparation was in general similar to that used by Mathëus, but his procedures were considerably modified. The series of reactions

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