explain the unfortunate results that have followed the clinical use of the drug; the persistent increase in intra-uterine pressure acting deleteriously on the child, and also tending to produce perineal lacerations or even uterine rupture. That such results invariably follow the use of pituitary solution is disproved by the many cases where the administration of the drug does no harm; the fact remains, however, that in patients where all the indications were present and where the attendant possessed undoubted skill, the employment of pituitary solution has, too often, led to serious or even fatal results to mother, child, or both.

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THE ASSAY OF MINNESOTA AND OTHER SAMPLES OF DIGITALIS.*

BY E. L. NEWCOMB AND E. B. FISCHER.

It has been customary for us to record each year the results of the assay of digitalis produced in Minnesota. These results are not reported to the Association each year, but we have attempted to present them all at one or another of the meetings. This year the results obtained in 1922, 1923, and 1924 crops are given; these include not only the assay of the official digitalis, but also of the leaves of *Digitalis lutea*, which we are growing in considerable quantities. The summary for many tests made each year are reported.

The U. S. P. official method is the biologic one, involving the use of frogs. The method in the present Pharmacopœia will be included in U. S. P. X. This official method has been used by us for a number of years, and determines the toxicity of the drug very well. Many pharmacologists believe that the official assay method probably is not a true index of the therapeutic value of digitalis; it is a test of the amount required to kill a frog. Some hold that the toxicity is directly comparable with the therapeutic value of the drug; work which others have done shows it does not always indicate the true therapeutic value of samples of digitalis.

In our work we have been endeavoring to develop a method which would more accurately measure the therapeutic value of the digitalis. Its general effect is to lower the rate of the pulse and increase the blood pressure. If we can develop a method which will accurately give us the measurement of the percentage of reduc-

* Read before joint meeting Northwestern Branch, A. PH. A., and Minnesota Pharmaceutical Association.

tion of the pulse, or the increase in blood pressure, we will have a measure of the therapeutic quality of digitalis.

There are many things that will kill animals used for biological work besides digitalis. One might take digitalis which contains certain impurities, even in minute quantities, and produce the death of the frog with heart systole, but such would not be a measure of the therapeutic value of the digitalis.

Another method used in standardizing digitalis is the guinea-pig method. Gold fish have been used; a certain dilution is placed in a given quantity of water in which they are swimming, and the gold fish will rapidly succumb. In addition, cats have been used for standardizing digitalis. The method of employing the cat, which was first used in Europe some years ago and introduced into this country by Dr. Hatcher, of Cornell University, involves the introduction of digitalis in the form of an infusion or dilution of the tincture into the femoral vein. As used by Dr. Hatcher and other workers since, it was a toxicity method where the minimum amount required to produce death within one hour or longer period of time was considered as the unit of activity of the digitalis.

During the war, we used several hundred cats in standardizing the drug supplied by the College of Pharmacy to the Army and Navy both in this country and abroad. We made quite a good many observations. We found it was possible to determine a certain percentage of reduction of the rate of the pulse, but there were difficulties because of the normal variation in the cat's pulse; animals will vary just as human beings in normal pulse rate. The variation in the cat is apparently more than in the human being, and the animal is likely to show quite a variation under the test.

During recent years we have been studying that particular factor in an endeavor to fix, if possible, a normal pulse rate for the cat for a given experiment. If we can do that and then apply our digitalis, and through its action arrive at the reduction which that drug causes, we can easily calculate a percentage reduction of pulse rate for that given sample.

In recent years we have been studying the pulse of the cat under the influence of ether for a longer period before beginning the injection of the digitalis. We are beginning to secure some figures which we hope will enable us to evolve a method by means of which we can express the activity of a sample of digitalis—not simply the amount required to kill the cat, but showing the percentage reduction of the pulse rate.

All of these tests involve the use of more than one animal, because of idiosyncrasy and the variation due to the influence of other factors. The more animals one uses, the more accurate is the final result. We are not ready to give a method for determination of percentage of pulse reduction, but we are working along that line. We mention it at this time because our studies do indicate we need something more than the toxicity test for digitalis. Many workers have found samples which were not potent or exceedingly toxic, and yet gave splendid reduction in pulse. There appears to be a therapeutic quality which is not so toxic as the other principles.

Digitalis is a very active agent in reducing the pulse rate of the cat. The different glucosides present similar properties, and digitoxin is more toxic than the other glucosides. Whether those samples which bring about reduction of the pulse

are not toxic, is based on the difference in the proportion of these glucosides—we do not know; but it would seem to be a logical explanation.

Our present assay methods are not as satisfactory as we wish them to be; studies on other species of digitalis are being conducted. *Digitalis lutea* is being employed in the work to a considerable extent; it compares favorably in all methods of assay with the official leaf. The plant does not yield as large an amount of leaves per plant as *Digitalis purpurea*. Earlier, it was supposed that *Digitalis lutea* had advantages over *Digitalis purpurea* because it was not so emetic; later work has not proved that claim. The emetic properties are due, primarily, to digitoxin which acts directly on the vomiting center. The work of earlier pharmacologists on this point has been criticized because they made their observations on the operating boards, and under the influence of ether, under which conditions animals rarely will show emetic symptoms, even when strong emetic drugs are administered. It is doubtful whether *Digitalis lutea* really possesses distinct advantages over the official product.

The results in 1922 on the carefully dried leaves of *Digitalis purpurea* showed the minimum lethal dose administered by the cat method, intravenously, was 77 mg. per kilo of cat. This represents an average of the whole series of tests.

In 1923, the minimum lethal dose was 76 mg. on a series of cats.

In 1924 on a series of animals it was 72 mg.—slightly more active than preceding years.

ASSAY OF MINNESOTA AND OTHER SAMPLES OF DIGITALIS.

Digitalis purpurea No. 40 powder—University of Minnesota 1923 Crop.

	Weight.	Sex.	
Cat No. 1	1.920 kilos	Female	80.7 mg. per kilo
Cat No. 2	2.550 kilos	Male	66.2 mg. per kilo
Cat No. 3	1.435 kilos	Male	84.0 mg. per kilo
Cat No. 4	1.850 kilos	Male	73.3 mg. per kilo
		Average.....	76.0 mg. per kilo

Digitalis purpurea No. 40 powder—University of Minnesota 1924 Crop.

	Weight.	Sex.	
Cat No. 1	4.155 kilos	Male	74.6 mg. per kilo
Cat No. 2	2.640 kilos	Female	81.1 mg. per kilo
Cat No. 3	2.160 kilos	Male	60.0 mg. per kilo
		Average.....	71.6 mg. per kilo

Digitalis lutea No. 40 powder—University of Minnesota 1924 Crop.

	Weight.	Sex.	
Cat No. 1	1.695 kilos	Male	80.0 mg. per kilo
Cat No. 2	1.695 kilos	Male	71.4 mg. per kilo
Cat No. 3	1.305 kilos	Male	67.8 mg. per kilo
		Average.....	73.1 mg. per kilo

Minnesota Digitalis—Grown near Lake Minnetonka.

Digitalis lutea No. 20 powder—1923 Crop.

	Weight.	Sex.	
Cat No. 1	3.580 kilos	Male	65.8 mg. per kilo
Cat No. 2	2.310 kilos	Female	63.0 mg. per kilo
Cat No. 3	2.980 kilos	Female	58.0 mg. per kilo
		Average.....	62.3 mg. per kilo

Digitalis lutea No. 40 powder—1924 Crop.

	Weight.	Sex.	
Cat No. 1	3.225 kilos	Male	76.0 mg. per kilo
Cat No. 2	2.605 kilos	Female	79.4 mg. per kilo
Cat No. 3	1.505 kilos	Male	74.0 mg. per kilo
		Average.....	76.5 mg. per kilo

Digitalis purpurea No. 20 powder—1923 Crop.

	Weight.	Sex.	
Cat No. 1	2.210 kilos	Male	74.4 mg. per kilo
Cat No. 2	2.825 kilos	Female (n. p.)	60.0 mg. per kilo
Cat No. 3	2.750 kilos	Female (n. p.)	68.0 mg. per kilo
		Average.....	67.5 mg. per kilo

Digitalis purpurea No. 100 powder—1923 Crop.

	Weight.	Sex.	
Cat No. 1	2.010 kilos	Male	85.5 mg. per kilo
Cat No. 2	4.655 kilos	Male	75.2 mg. per kilo
	Coarse powder.		
Cat No. 3	2.930 kilos	Male	71.0 mg. per kilo
		Average.....	77.2 mg. per kilo

We have had difficulties in obtaining a sufficient number of test animals. While three of the four animals run on the 1924 test gave close results, the fourth was considerably off, apparently due to the use of an animal entirely abnormal to the action of digitalis. With a larger number of test animals on hand, we would have repeated this test, because there is quite a disparity between the figures 72 and 76.

We are studying this whole question with a view of determining whether seasonal variations—temperature, moisture, etc.—have any bearing on the variation in the activity of the drug. It would appear there is not very much variation, although this work, in order to draw conclusions, must extend over a period of years, where we have exceedingly dry periods and exceedingly hot or cold summers. Heretofore we reported on the collection and assay of digitalis by months for the entire year. Our reports show there is little variation between the middle of the summer, when the plants reach maturity, and the first of December, when they are frozen and covered with ice. Freezing the leaves does not injure them.

The use of digitalis by the pharmacist will undoubtedly continue for many years. It is one of our most important drugs.

We want to say a word about the making of the official infusion. The old method was to use the bruised leaf. Where the word "bruised" came from we do not know. Apparently it has been handed down from the time when the apothecary used the fresh leaf. If you try to bruise the dry leaf, you produce a powder; the word is not properly used; its application originally was apparently to bruise a fresh, green leaf, and then apply hot water. If one attempts to make an infusion from the whole drug, which is composed of broken parts of the leaf, it will be found in weighing out three or four grams that it is difficult to get a uniform sample—there are portions of the midrib and the petiole and the lamina part. The midrib and petiole contain only one-fourth of the activity of the lamina portion of the leaf. Two or three pieces of the petiole will weigh more than remaining part required to make up the weight.

It is exceedingly difficult for any pharmacist to take a broken or dried digitalis leaf and weigh out five or six lots and have them represent the same amount of

activity of the digitalis. If you are going to put out an infusion of digitalis, uniform in therapeutically active constituents each time you fill a prescription, you must have the drug in such a form that you can withdraw uniform samples.

In the new Pharmacopœia instead of specifying that one shall use so much of the bruised digitalis, it is directed that one shall use so much of the No. 60 powder. One may withdraw typical samples, each of which will represent practically an identical amount of the therapeutic qualities in the drug.

The old method also directs to strain the infusion. If one uses a No. 60 powder, the strainer must be very fine. The best procedure is to weigh out the digitalis in powdered form; introduce hot water and allow it to stand, and then filter it. We do not know whether the change from the whole drug to the powdered drug is going to make the problem of the pharmacist in supplying the official infusion any more difficult than to use the whole drug. Certainly every pharmacist can quickly tell the difference between a nice whole digitalis leaf and one which is of inferior quality.

In the future you are going to have the powder to work with. What will you do about determining the quality of the powder? We want to issue a word of warning about powdered digitalis which is brown or gray. If prepared from select green leaf, it should be of a nice, rich, green color. If it is, you may rest assured a preparation made from it, providing it has been standardized, will give an infusion which will give the result the doctor has a right to expect.

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THE TANNIN OF RHUS GLABRA.*

BY JOSIAH C. AND BERTHA L. DEG. PEACOCK.

The title "Rhus Glabra," previously used by the United States Pharmacopœia to designate the fruit of that plant, is now employed for the same purpose in the National Formulary by which authority the drug is recognized.

That *Rhus glabra* contains an astringent principle—a tannin—has long been known, and on the recognition of this quality it has found use.

This drug has been the subject of several investigations, references to which were given by Henry Kraemer in *American Journal of Pharmacy* for September 1913, in an article explaining that in commerce the fruit of *Rhus glabra* was being replaced by the fruit of *Rhus typhina*, and, in which paper, besides summarizing the reports of those who had contributed to an understanding of the constituents, he gave means for distinguishing these fruits by examining their hairs under the microscope.

As mentioned by Kraemer, Watson in 1853 (*Amer. Jour. Phar.*, p. 193) published the results of his examination of the fruit of *Rhus glabra*, substantiating earlier statements by Cozzens and Rogers, the first of whom was concerned chiefly with establishing the presence of gallic and malic acids, while the latter pertained more to determining the form in which the malic acid occurs.

It is mainly on the statements of Watson that the references to the constituents of *Rhus glabra* found in the books which American pharmacists study and consult are based.

* Read before Pennsylvania Pharmaceutical Assoc., Washington, Pa., June 16–18, 1925.