T H E S I S

on

"The Potency of Oregon Digitalis"

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CONTENTS

Introduction:

The activity of native (uncultivated) digitalis has long been a subject of controversy. This investigation was prompted by numerous inquiries concerning the comparative activity and potency of the wild-growing Oregon foxglove and the cultivated plants used in commerce. In order to ascertain the potency of Oregon foxglove, monthly collections of the digitalis leaves were made in Lincoln County, Oregon. From these collections, tinctures of digitalis were prepared conforming to the formula and directions of the United States Pharmacopoeia X. The preparations were then biologically assayed by the Official "One-hour" Frog Method to determine their potency.

Chemical tests were made of the respective tinctures to check and determine the total glucosidal contents of each in comparison to the results obtained from the biologic assays.

History:

The real professional use of digitalis (1) is relatively recent: nevertheless, this plant was known to Welsh physicians as early as 1250 A.D. The herb was used internally in the treatment of various diseases and externally it was applied to wounds and ulcers. Up to the decade of 1775 to 1785, this drug plant, although recognized in several pharmacopoeias, remained an unimportant medicine. During this period there lived in England a young physician by

the name of Wm. Withering whose home was in Birmingham. It came to his attention that an old woman in Shropshire was preparing an herb concoction alleged to be of value in the treatment of dropsy. When the Dean of Brazenose College, Oxford, was ill with dropsy, the medical attention at that time failed to restore him to health. Some of the old woman's remedy was administered to the Dean, and in due time he was greatly relieved of his dropsical condition. Withering, although credulous about the value in this herb concoction, decided to make an investigation of the matter. Upon examination of the formula of the herb-tea, he found that foxglove was among the ingredients employed. With this knowledge, he proceeded to investigate the activity of the different parts of the plant by administeringthe preparation to his patients. This keen observer discovered that the leaf of the plant was the vital part which possessed the cardinal medicinal virtues of the plant. To further his study, he administered a tea prepared solely from the digitalis leaves to his patients for a number of years, in his treatment for dropsy, but at that time he did not fully realize that the tea from the leaves had a definite heart-tonic action. In 1785 after ten years of experimentation, Withering published his epoch-making paper entitled "An Account of the Foxglove and Some of Its Medical Uses with Practical Remarks on Dropsy and other Diseases." This was his first paper ever published on the foxglove, but the vital point is that this

keenly observing Englishman was responsible for the first rational use of the now time-honored drug digitalis.

After the preliminary works of Withering, increasing emphasis was placed upon the action of digitalis as a heart tonic. In 1779, John Ferriar observed the relationship of the circulatory and diuretic effects of this drug, and in 1800 he reported that extractions from the leaves of the foxglove, when administered,"Furnished us with means of regulating the pulses to our wishes and of supporting a given state of velocity as long as we may judge it proper." Prompted by the wisdom of their predecessors, the modern generation of scientists such as McKenzie, Cushny, Brody, Hatcher, Robinson, Eggleston, Sollman, etc., have continuously delved in the digitalis therapy. Through the efforts of this succession of investigations, digitalis today is a prominent remedy in the treatment of various cardiac disturbances.

Botanical Aspects:

The official United States Pharmacopoeia X. digitalis (2) is obtained from the plant Digitalis purpurea Linne (Scrophulariaceae). There are many other varieties of digitalis grown, but since the International Conference at Brussels in 1902, the purpurea species of foxglove is internationally recognized as the official plant of digitalis and its preparations.

The official plant name (3) is derived from two Latin words, "digitalis" meaning "finger" and referring to the finger-shaped corolla. This was named by Tusche in 1542 after

finger-shaped corolla. This was named by Fusha in 1542 after the German name of the plant "Fingerhut". The species of the plant is derived from the word "purpurea" meaning "purple", from the color of the flowers. As all plants have a common name for which the layman could recognize them, digitalis is of no exception. Some of the many synonyms of Digitalis purpurea L. are Foxglove, Fairy Glove, Fairy Cap, Fairy Finger, Fairy Thimble, Fairy Bell, Ladies Glove, Throatwort, Flopdock, Digitale (It.), Dedalera (Sp.), Dedaleira (Port.) Herva Dedal (Port.), Fingerhutblater (Germ.), Doigtier, Feuille de Digitale pourpree (Fr.), and many others.

Washburn and Blome described this plant (3) as a beautiful herbaceous plant with a biennial or perennial root which sends forth the first year a number of large tuft leaves, and the following seasons a single erect, leafy stem terminating in a beautiful spike of purplish flowers. The flowers are arranged along on one side of the stem in a pendulous manner, and are either whitish or purplish crimson color. They are marked within with dots, which doubtless serve to attract the bees, since they are continually favored by their visits. This plant is found growing both wild and cultivated in Central and Southern Europe. In the United States, it is found chiefly in the Western Coast region, all the way from Vancouver Island to California. In Oregon it grows abundantly along the western part of the State, but more especially in Lincoln and Coos Counties.

The United States Pharmacopoeia X. specified that the official part of the Digitalis purpurea L. used in medicine is the dried leaf (2) which in the form of a tincture, properly diluted and injected into the ventral lymph sac of the frog (Rana pipens), has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systolic) not exceeding 0.006 cc. of the tincture equivalent to 0.00,000,05 Gms. of ouabain, for each gram of body weight of frog. The official leaves should contain not more than 2% of brown leaves, stems, flowers, or foreign organic matter, and yield not more than 5% of acid soluble ash.

Dried digitalis leaves (2) when unground are more or less crumpled or broken; blade ovate to ovate-lanceolate, about 25 cm. in length and contracted into a winged petiole; margin crenate or irregular; thin, lower surface densely pubescent, upper surface wrinkled, finely hairy; venation conspicuously reticulate, the midrib and principal veins broad and flat, and the lower veins continued into the wings of the petiole; color of the upper surface dark green, of the lower surface grayish from the dense tomentose pubescence, the large veins often pruplish; odor slight when dried peculiar and characteristic when moistened; taste, very bitter.

There are many other official and non-official drugs which possessess a heart-tonic effect similar to the digitalis species in their physiological actions and these are all clas-

sified under the general heading of "The Digitalis Group".

The more important members of this group are Digitalis (Digitalis purpurea); Strophanthus (Strophanthus hispidus or kombe); Squill (Scilla maratima); and Apocynum (Apocynum cannabinum). Others which are of less importance are Christmas Rose (Helleborous niger); Lily-of-the-Valley (Convallaria majalis); Pheasant Eye (Adonis vernalis); Upas Tree (Antarias); Oleander (Nerium). These allied plants all have a general similarity in cardiac action, belong to the Phytokingdom, but likewise similar action is noticed in substances isolated from species of the animal kingdom. Abel and Macht (4) in 1911 were able to isolate a principle from the "Parotid" glands of the Bufa agua, the tropical toad. They called this new principle "Bafogen", which has a similar effects on the cardiac muscles as those of digitalis and its allied plants. In 1920, Handovsky (5) isolated an alkaloid "Bufotenin" from the skin of the common toad. This product possessed the power to stimulate the vagus center but depressed the respiration, brain, and cord.

In the inorganic chemical field we find that barium salts and alkaline hydrates, and even normal saline solution will produce cardiac actions similar to those of digitalis. Although all these cardiac stimulants exist, digitalis was the earliest cardiac stimulant put into practical and clinical usage, and is still the most common cardiac tonic in the medi-

cal.profession. Most of the members of the so called "Digitalis Group", chiefly those found in the inorganic chemical field, priduce superficial resemblances to the true digitalis (5).

Cultural Comparison and Problems:

Ever since the medical profession has adopted the use of digitalis as a cardiac tonic, much of the supply of the drug has been shipped into this country from Europe, especially from Germany and Australia. During the recent World War (1913-1918) the supply from the foreign countries was greatly diminished and drug firms had to seek new sources for this valuable drug. In 1914, Wilbert (6)(7) in his report to the United State Public Health Service called the attention of the professional men to a hitherto neglected source for obtaining digitalis plants, namely, in the collection of the wild-growing plants which have escaped from cultivation and have now become a "weed" in various sections of this country. Although the recognition of this fact was slow in showing results, throughout the later years of the World War, a great increase in the usage of local digitalis was noticed. In answering to the call of Wilbert in 1914, numerous manufacturing firms today have their own botanical farms where digitalis and many other valuable drugs are cultivated for commercial use.

With the utilization of home grown digitalis; the variations due to the influences of climate, soils, age, etc. in affecting the yield and potency of the drug should be alluded

to. Miller (8) claims that very little affect could be attributed to the uses of commercial fertilizers such as sodium sulphate, superphosphate, sodium nitrate, and others upon the digitalis plants. He says "that in the cultivation of digitalis, non-fertilization of plants does not lessen the plant toxicity in comparison with those having fertilizers to promote growth." Likewise he also claims"that cultivation will not lessen the toxicity as a whole." In Canada, Sparks (9) found that the usage of sodium nitrate as a fertilizer increased the yield of the leaf about 15 per cent per acre, and increased the glucosidal content 40 per cent over that of the unfertilized plants. Daulphinee (10) in his experiments found that ordinary cow manure was the most effective fertilizer and his increases were twice as great in comparson with other commercial fertilizers, but his tabulations do not show an increase in the glucosidal content of the leaf. In Italy, Cippini (11) cultivated five different samples of digitalis for a period of six years (1915-1921) and found that these tame or cultivated plants had equivalent potency in comparison with those of the wild drug plant. Pater (12) went a step further and found that best results were obtained when the seeds where sown in November and harvested the following September. Hammer (13) found that there is no reason for preferring wild digitalis to the cultivated drug. He says "that the diminution of the activity of the drug does not occur as early as frequently assumed." Focke (14) found that the cult-

ivation of wild digitalis is more toxic than the cultivated plants and that the second year's growth is more potent than that of the first year plants.

There seems to be a belief that the leaves of the second year or the older plants are more potent than the leave of the first-year plants, but recent investigations have placed this belief in a doubtful position. This phase of the cultivation problem of digitalis is somewhat a new field and many substantiated proofs have been given by both sides in the determination of the question. Miller and Baker (15) collected first-year leaves and found that these were nearly as active as leaves of the second-year plants from the wild drug, and that conservatory conditions do not materially lessen the activity. On the other hand, Straub (16) found that the second-year leaves are better than those of the first year, and at the same time pointed out that the use of fertilizer will also increase the yield. The author in testing the potency of Oregon digitalis found that the variation of both the plants is very slight. Nothing definite could be said as there are many factors yet to be investigated.

Phyto-Chemical Constituents:

Digitoxin Group: During the period of 1827-1869, German and French pharmaceutical chemists attempted to isolate the active principles of the drug, which consists of glucosides

allied to saponin in composition and actions but more specialized in the location of their effects and also more absorbable. In 1869, Nativelle (1) Succeeded in isolating from digitalis leaves a crystalline principle which he call "Digitaline". Later this extracted principle was named "Digitoxin" (1875) by Schmiedeberg and today it is known by the latter name. This principle is the most important of all the glucosides found in the digitalis leaves. It is extracted by alcoholic and hydro-alcoholic solvents from the leaves only as it is not found in the seeds. According to Kiliani (17) this glucoside has the empiric formula $C_{34}H_{54}O_{11}$, and that on hydrolysis (5) it would yield a hexose (digitoxose) and the crystalline digitoxigenin. In the chemical identification, Nelson (18) found that on the application of Keller's test, digitoxin would give a dirty brown green zone at the point of contact, while the upper layer of glacial acetic acid solution becomes intense blue-green and the lower sulphuric acid becomes brown or perhaps red after a day or more. Nelson also investigated the quantitative composition of this glucoside and he found that this contains C. 63.95%; H. 8.4%; O. 27.61%; and has a melting point of 240°C. (dry).

Gitalin Group: Another glucosidal group is the gitalin group. These are amorphous principles, soluble in water and more soluble in chloroform. Kraft in 1912 thought he had isolated the pure gitalin, but Kiliani and Rosenthaler (1)

found that his isolated product was a mixture of principles and not a pure substance. Nelson (18) found that his principle contains 65.21 per cent Carbon, 8.69 per cent Hydrogen, 26.1 per cent Oxygen, and also that on application of the Keller's test it gives a fiery red zone. The melting point of this is 217°C. and on hydrolysis, gitalin yields digitalose and digitaligenin which on reduction will in turn gives hexahydrodigitaligenin ($C_{24}H_{38}O_3$).

Digitalein Group: These are ater-soluble glucosides which occur both in the seeds and in the leaves of the digitalis plants, and have the empirical formula (16) $C_{22}H_{38}O_9$. It has a melting point of 230-232°C. and is soluble in alcohol but insoluble in ethereal solvents. With Keller's test (17) it gives similar results as the digitoxin; i.e., a fiery red zone is formed at the point of contact while the upper acetic acid solution becomes yellow to brown and the sulphuric acid to golden yellow red and finally to violet.

Digitonin Group: These comprise the more typical water soluble saponins; digitonin, which is isolated from the digitalis seeds, and gitin, which is from the leaves. These (5) generally do not have the typical digitalis affects on the heart, but probably their chief use is the modification of the solubilities of other principles. These occur in both the amorphous and crystalline forms having a melting point of 235°C. and an empirical formula $C_{50}H_{26}O_2$. With Keller's test

they give a rose color zone (18) and on hydrolysis (19) will yield digitogenin ($C_{26}H_{42}O_5$) and two glucose plus two molecules of galactose. Although they do not produce the digitalis effects, they do however, contain irrtant and hemolytic property of the saponin group.

These foregoning principles are the chief constituents found in the digitalis plants, but as to the minor constituents, they contain manganese, volatile oil, fixed oil, gum, starch, sugar, chlorophyll, inosit, pectin, coloring matter (red and yellow), digitalic (malic) acid, and ash (20).

Physiological Properties:

Before going into the detailed discussion of the physiological properties of digitalis, we should review the construction of the heart and the mechanism of circulation.

The heart is a hollow muscular cavity whose function is to maintain the circulation of blood throughout the body. This muscular cavity is divided into four chambers by two walls. The verticle wall divides the heart into right and left while the horizontal wall divides the heart into upper and lower portions. The upper chambers are termed auricle and the lower chambers are termed ventricles. Between these chambers are valves whose function is to prevent the back flow of blood once it passes through these valves.

The general route of blood circulation is that blood flows from the right auricle through the tricuspid valve into the right ventricle. The ventricle then contracts as the valve closes and the blood is forced through the pulmonary

valve closes and the blood is forced through the pulmonary artery into the lung where it is oxygenated. From the lung the newly oxygenated blood is carried by the pulmonary vein into the left auricle which in turn contracts and forces the blood through the mitral valve into the left ventricle. On closing of the mitral valve, the left ventricle will contract and force the blood through the aorta and into the general circulatory system.

Simultaneously on each auricle contractions, we hear a heart beat and after a brief pause of about 0.1 second, the contraction of the ventricle follows and the blood is forced out into the arteries. The contraction of the auricles or the relaxation period of the heart is called diastole and lasts about 0.4 second whereas the contraction of ventricle (systole) covers aperiod of about 0.3 of a second.

The cause of this rhythmic contractions of the heart has been a very deep problem of concern to the physiologist. It has been proposed (1) that this heart beat is due to a cardiac hormone called "automatin" but this have not been definitely prove. We do know, however, that there are two sets of nerve fibres which lead to the heart and that the stimulation of these will have marked affects on the heart beat. One of these nerves is the vagus or sometimes called the pneumogastric nerve which is really an inhibitory cardiac nerve. The other is the nerve from the sympathetic nervous system which stimulated the rate of heart beat. Each of

these nerves is automatically controlled and is beyond the modification of the will.

The bundle of His (1) has been found to be the chief area affected by the action of the digitalis glucosides. According to the physiologists this bundle of His is the canal for the transportation of quiverings which occur in a diseased heart. These quiverings pass through so rapidly that the ventricle which pumps the blood into the body respond very ineffecient to the auricle impulses; thus the irregular and ineffecient ventricular action of the heart causes a decided decrease in the amount of blood in circulation and contributes to the affection of dropsy. The administration of digitalis will protect the ventricle from rapid successions of auricular impulses and the ventricles are allowed sufficient time for their beats, thus again allowing the normal volume of blood to moventhrough the circulation with the results of dropsical condition being removed.

The action of digitalis (5) is divided into two main divisions: First, the local action which consists of primary irritation followed byfrequent paralysis of the sensory nerve endings. This irritation is not equally marked throughout the digitalis series of glucosides as digitoxin is more powerful in this respect while on the other hand digitalin may be injected subcutaneously without danger and often without pain.

Secondly, the digitalis series has a general action which comes after absorption of the drug. The symptoms involved then are due to the action of the drug on the Central Nervous System and also that of the heart. The action on the Central Nervous System consists in the stimulation of some of the nerve centres, which are independent of the action in the medullary and in many cases limited to the medulla oblongata. The action on the heart is the most important of all the actions possessed by digitalis, andit is this characteristic reaction which distinquishes digitalis and its allies from all other substances.

The effect of the cardiac action is exerted chiefly in three ways: first, it increases the tone of the heart by prolonging the systolic period; second, it prolongs the contraction by increasing the irritability; and thirdly, it slows up the heart by stimulating the vagus center along with other medullary centers.

When digitalis is administered in therapeutic doses, three well marked stages of reactions are observed upon the heart. First: the therapeutic stage is accompanied with a slight acceleration of the heart beat and a slight rise of blood pressure. Second: the inhibitory stage is accompanied by slow and irregular pulses, with greater prolonged contractions resulting in a decrease of output of blood. The differentiating point (21) between the first and the second

stage is that there is a weaker contraction of the auricles in the first stage and that the amount of blood expelled per minute in the first stage exceeds that of the second stage, the volume of blood expelled per minute being even less than before the drug is administered. Thirdly and lastly; is the toxic stage which is usually the result of excessive amount of the drug. This stage is accompanied with an increased rate of both auricular and ventricular contractions with the appearance of apparent extra systole. Finally there is a fluttering of the heart which ends in death.

The forgoing actions mentioned are the chief effects of digitalis and it allies, yet there still are other minor or secondary actions and therapeutic properties. Digitalis (20) also possesses properties as vascular stimulant, diuretic, motor excitant, paralyzant, anaphrodisiac, sedative, narcotic, and emetic; hence it is sometimes used in renal diseases, venous engorgement, dropsy, pneumonia, scarlet fever, congestive headache, delirium, hemorrhages, menorrhagia, rheumatic fever, spermatorrhea, pleurisy, pericarditis, chronic bronchitis, and epilepsy. Locally it is used for enlarged glands, abdominal and renal dropsy.

EXPERIMENTAL STATISTICS.

Seasonal Collections and Curing of Crude Drugs:

Monthly collection of leaves from first and second year plants of the wild-growing digitalis thriving in the counties

of Western Oregon were made. From these collection, a tincture was to be prepared according to the formula and method stated in the United State Pharmacopoeia X. and pharmacologically assayed by the official "One-hour" frog method, which is officially recognized in the United State Pharmacopoeia X. Due to the unusual weather throughout the entire Pacific North-west this year (1932-1933) vegetative growth was retarded, most of the digitalis plants were either frozen or killed, and new plants were late in blooming.

Digitalis leaves were collected in Lincoln County about forty-one miles south of Corvallis, Oregon, on the Corvallis-Newport Highway. This plant was identified by the Botanical Department of Oregon State College as Digitalis purpurea,L. As the United States Pharmacopoeia X gives the formula for the preparation of two official preparations, the tincture and the infusion of digitalis, the tincture was chosen for biological assay as it is used most extensively by medical practitioners and contains a definite strength (10%).

It is amzing to find after reviewing the literature of pharmacology, the large variety of methods of assay which have been devised for the standardization of digitalis. In the plant kingdom we find the usage of Lupinus albus by Macht and Krantze (22), while in the animal kingdom we find the usage of Paramecia, Dalphinia, gold-fish, frogs, and advancing along the line to guinea-pigs, cats, and dogs as

test objects for the assay of this cardiac tonic. The frog method is one of the oldest known method for assaying and the one adopted by the United State Pharmacopoeia; hence the writer chose this method in his assay of the potency of the native Oregon foxglove. There are a number of modifications of this frog method; the principal variation is in the period of time required for the drug to produce the systolic stoppage, but since the United State Pharmacopoeia X specifically recognized the "One-hour" method, this method was used in preference to all other methods.

Preparations of Digitalis Products:

After collection of the fresh drug, the leaves were immediately washed and dried at room temparature, and then finely ground. Fifty grams of the finely powdered leaves were used to prepared 500 cc. of the Official Tincture as directed in the United State Pharmacopoeia X.

The powdered leaves were first defatted with petroleum benzene, as the fatty material of the digitalis leaves is supposed to cause the nauseating reaction when the drug is administered, and that fat-free tinctures are less subject to deterioration than that from the original drug (23). After defattation, evaporation was allowed to proceed wuntil the odor of benzene had disappeared. The pwodered leaves were then macerated for six hours, packed firmly in a glass percolator and again macerated for twenty-four hours. The

second maceration was to allow the menstruum to permeate the small particles of the powdered leaves so as to extract all the glucosidal content which contains the active principals of digitalis. At the end of the maceration period, the drug was allowed to percolate slowly until 500 cc. by volume was acquired. The menstruum used for all preparations was in accordance with the United State Pharmacopoeia X specifications: four parts of alcohol (95%) to one part of distilled water. At the conclusion of percolation, the resulting product was placed in a glass-stoppered amber-colored bottle, and stored in a cool place, as (24) (25) heat and light will cause rapid deterioration of the active principals.

Biological Assay:

The method used for the determination of the potency of Oregon foxglove is the Official "One-hour" Frog Method. This method (26) principally consists in determining the minimum amount or dose of drug per gram body weight of animal that will cause permanent systolic standstill of the frog's ventricle at the end of exactly one hour. Throughout the experiments only healthy frogs of the species Rana Pipiens (common "grass" or Leopard" frogs) weighing between 20-35 grams were used. Frogs showing signs of illness or signs of a disease called "red-leg" were immediately separated from the healthy frogs. Temperature was likewise kept constant (20°C.) as this is one of the chief factors which

would cause variations in the results.

The day before the frogs were to be used, a sufficient number where taken from the storage tank and placed in another tank, the temperature of which is kept approximatly at 20°C. One hour before assay, they are weighed to within 0.5 gram and placed in separate individual cages and again kept at the uniform temperature of 20°C.

As the original prepared tincture contains too strong an alcoholic content to give good results, it is diluted with distilled water until it contains no more than 20 per cent of alcohol by volume. The dose is then calculated according to the weight of individual animals, and accurately measured to a hundredth of a cc. in a glass syringe, and injected directly into the anterior lymph-sac of the frog. The injections were made with due care so as not to puncture the skin, as the skins of the cold-blooded frogs are not so elastic as those of the warm blooded mammals.

A series of frogs was used for each soltuions to be assayed. The frogs were injected with 9/10, 10/10, 11/10, 12/10, respectively, of the standard dose (10/10) of the preparation being tested, for each gram body weight of the animal, until the approximate strength of the preparation is ascertained. A second and third series of frogs are again in like manner with doses ofsmaller variations than the first series until there is a confirmation of the results.

After the injection the animals were returned to their respective cages and the temperature checked to be constant at 20°C. About fifty-eight minutes from the time of injection each animal was pithed, the heart exposed, and its condition examined. The pharmacopoeia (2) gives the correct end reaction of the assay as follows: "The correct end-point at the expiration of exactly sixty minutes from the time of injection, the ventricle of the heart must be in systolic standstill while the auricles are highly dilated. Following mechanical stimulation, feeble contraction may occur in the auricles and localized contraction of the ventricle, but no general contraction is allowable. If on opening, the lymph sac shows any unabsorbed drugs, the animal is discarded and is not considered in the results obtained."

The dose found in this series of injections is then compared with the dose of the standard ouabain solution, which is similar ascertained upon another series of frogs of the same lot. From these two determined doses, the percentage strength of the unknown tincture of digitalis is determined by the following formula:

Standard dose of ouabain	:	Found dose of ouabain	::	Standard dose of drug being assayed	:	Nec. dose of unknown.

The standard dose for both of these as specified in the United State Pharmacopoeia X (2) is that "the tincture of digitalis injected into the ventral lymph-sac of a frog

has a minimum systolic dose of not less than 0.0055 cc. and not more than 0.0065 cc., equivalent to not less than 0.00,-000,046 gram and not more than 0.00,000,054 gram of ouabain for each gram of body weight of frog."

The purpose of using ouabain is that it has a similar heart action as that of digitalis; hence it is used in the determination of the degree of sensitiveness of the frogs to the drug as the sensitiveness of frog varies throughout the year. Thus, if a frog series is especially sensitive to the ouabain systolic dose, then it will likewise be hypersensitive to digitalis. In other words, the ouabain is used chiefly to standardize the series of frogs for the digitalis assay.

Laboratory Results:

The following tabulations give the results as obtained by actual experimentation. Each table shows a series of frogs which were used to standardize the particular age and the months of collection of the digitalis leaves under the following headings: (1) The weight of the frogs in grams; (2) The normal dose as calculated by the weight of the frogs to be tested; (3) The series of doses with which these frogs are injected; (4) The actual dose of injection as calculated from the series of dosage; (5) The results obtained at the end of sixty minutes.

Frog Assay Results:

Table I.

First Year Plants of November Collection.

Dilution of Tincture 1:5

Wt. of Frogs in grams.	Normal Dose.	Series of dosage.	Actual Dose	Results.
(1) 25.1	0.753 cc.	12/10	0.904 cc.	Alive.
(2) 24.1	0.723 cc.	13/10	0.980 cc.	Alive.
(3) 23.4	0.702 cc.	14/10	0.983 cc.	Dead.
(4) 25.0	0.750 cc.	13/10	0.975 cc.	Alive.
(5) 22.0	0.660 cc.	14/10	0.910 cc.	Dead.
(6) 24.8	0.743 cc.	15/10	1.114 cc.	Dead.

The relative potency of this series is 35.71 per cent as dtermined by comparison with ouabain as standard.

Table II

Second Year Plants of November Collection.

Dilution of Tincture 1:5

Wt. of Frogs in grams.	Normal Dose	Series of dosage.	Actual Dose	Results.
(1) 28.7	0.861 cc.	12/10	1.033 cc.	Alive.
(2) 24.6	0.738 cc.	13/10	0.959 cc.	Alive.
(3) 23.7 -	0.711 cc.	14/10	0.995 cc.	Alive.
(4) 25.8	0.774 cc.	15/10	1.160 cc.	Dead.
(5) 26.0	0.779 cc.	14/10	1.090 cc.	Alive.
(6) 23.2	0.696 cc.	15/10	1.044 cc.	Dead.
(7) 27.5	0.824 cc.	16/10	1.318 cc.	Dead.

The relative potency of this series is 30 per cent as determined by comparison with ouabin as standard.

Table III

First Year Plants of December Collection.

Dilution of Tincture 1:5

Table III.

First Year Plants of December Collection.

Dilution of Tincture 1:5

Wt. of Frogs. in grams.	Normal Dose.	Series of dosage.	Actual Dose.	Results
(1) 24.4	0.732 cc.	14/10	1.024 cc.	Dead.
(2) 25.8	0.774 cc.	15/10	1.161 cc.	Dead.
(3) 26.2	0.786 cc.	16/10	1.238 cc.	Dead.
(4) 28.4	0.852 cc.	12/10	1.020 cc.	Alive
(5) 24.9	0.746 cc.	13/10	0.969 cc.	Dead.
(6) 26.4	0.792 cc.	12/10	0.950 cc.	Alive
(7) 27.9	0.836 cc.	13/10	1.086 cc.	Unabs.
(8) 25.7	0.771 cc.	14/10	1.079 cc.	Dead.
(9) 30.1	0.902 cc.	12/10	1.082 cc.	Alive
(10) 19.6	0.587 cc.	12/10	0.704 cc.	Alive
(11) 25.1	0.752 cc.	13/10	0.977 cc.	Dead.

The relative potency of this series is 38.46 per cent as determined by comparison with ouabain as standard.

Table IV

First Year Plants of April Collection.

Dilution of Tincture 1:4

Wt. of frogs in grams.	Normal Dose.	Series of dosage.	Actual Dose.	Results
(1) 30.45	0.732 cc.	15/10	1.098 cc.	Dead.
(2) 26.0	0.624 cc.	20/10	1.248 cc.	Dead
(3) 28.3	0.678 cc.	12/10	1.017 cc.	Alive
(4) 23.9	0.574 cc.	13/10	0.746 cc.	Dead.
(5) 22.7	0.545 cc.	14/10	0.773 cc.	Dead.
(6) 18.6	0.445 cc.	12/10	0.534 cc.	Alive
(7) 23.6	0.565 cc.	13/10	0.735 cc.	Dead
(8) 23.6	0.566 cc.	14/10	0.793 cc.	Dead.

The relative potency of this series is 38.46 per cent as determined by comparison with ouabain as standard.

Table V.

Ouabain Solution As Standard.

Dilution of Solution
1:10,000

Wt. of frogs in grams.	Normal Dose.	Series of dosage.	Actual Dose.	Results
(1) 28.9	1.445 cc.	10/10	1.440 cc.	Dead.
(2) 27.1	1.355 cc.	11/10	1.48 cc.	Dead.
(3) 19.7	0.985 cc.	9/10	0.88 cc.	Dead.
(4) 22.9	1.145 cc.	10/10	1.145 cc.	Dead.
(5) 25.3	1.265 cc.	8/10	1.012 cc.	Dead.
(6) 32.5	1.625 cc.	7/10	1.13 cc.	Dead.
(7) 22.4	1.110 cc.	6/10	0.834 cc.	Dead.
(8) 27.8	1.390 cc.	5/10	0.555 cc.	Dead.
(9) 26.8	1.340 cc.	4/10	0.536 cc.	Alive
(10) 28.6	1.430 cc.	4/10	0.572 cc.	Alive
(11) 25.9	1.293 cc.	5/10	0.646 cc.	Dead.

This series of ouabain solution is used as a standard for the tinctures under Tables I, II, III, and IV., resulting 0.00,000,025 gram per body weight of frog.

Table VI.

First Year Plants of June Collection.

Dilution of Tincture 1:5

Wt. of frogs in grams.	Normal Dose.	Series of dosage.	Actual Dose.	Results
(1) 18.8	0.564 cc.	6/10	0.338 cc.	Alive
(2) 18.7	0.561 cc.	7/10	0.393 cc.	Dead.
(3) 29.6	0.888 cc.	5/10	0.444 cc.	Alive
(4) 33.9	1.017 cc.	6/10	0.610 cc.	Alive
(5) 30.2	0.906 cc.	7/10	0.634 cc.	Dead.

The relative potency of this series is 85.71 per cent as determined by comparison with ouabain as standard.

Table VII

Second Year Plants of June Collection

Dilution of Tincture 1:5

Wt. of Frogs in grams.	Normal Dose.	Series of dosage.	Actual Dose	Results
(1) 16.0	0.475 cc.	8/10	0.384 cc.	Dead.
(2) 18.1	0.543 cc.	9/10	0.489 cc.	Dead.
(3) 25.8	0.774 cc.	10/10	0.774 cc.	Dead.
(4) 18.9	0.567 cc.	11/10	0.624 cc.	Dead.
(5) 21.6	0.648 cc.	7/10	0.453 cc.	Dead.
(6) 22.4	0.671 cc.	6/10	0.402 cc.	Alive
(7) 29.9	0.897 cc.	5/10	0.449 cc.	Alive.
(8) 18.5	0.555 cc.	6/10	0.333 cc.	Alive
(9) 20.6	0.618 cc.	7/10	0.433 cc.	Dead.
(10)21.6	0.648 cc.	8/10	0.518 cc.	Dead.

The relative potency of this series is 85.71 per cent as determined by comparison with ouabain as standard.

Table VIII

First Year Plants of July Collection.

Dilution of Tincture 1:5

Wt. of Frogs in grams.	Normal Dose	Series of dosage.	Actual Dose	Results
(1) 30.9	0.927 cc.	5/10	0.464 cc.	Alive
(2) 37.8	1.134 cc.	6/10	0.680 cc.	Dead.
#3) 30.3	0.909 cc.	7/10	0.636 cc.	Dead.
(4) 31.6	0.948 cc.	6/10	0.569 cc.	Dead.
(5) 34.5	1.035 cc.	4/10	0.414 cc.	Alive
(6) 38.5	1.155 cc.	5/10	0.578 cc.	Alive

The relative potency of this series is 100 per cent as determined by comparison with ouabain as standard.

Table IX

Second Year Plant of July Collection

Dilution of Tincture 1:5

Wt. of Frogs in grams.	Normal Dose	Series of dosage.	Actual Dose	Results
(1) 27.7	0.8310cc.	5/10	0.416 cc.	Dead.
(2) 22.0	0.650 cc.	6/10	0.390 cc.	Dead.
(3) 23.8	0.714 cc.	7/10	0.500 cc.	Dead.
(4) 25.2	0.756 cc.	4/10	0.302 cc.	Alive
(5) 20.9	0.627 cc.	5/10	0.314 cc.	Alive
(6) 21.6	0.648 cc.	6/10	0.389 cc.	Dead.
(7) 19.3	0.579 cc.	4/10	0.232 cc.	Alive
(8) 21.0	0.630 cc.	5/10	0.315 cc.	Alive
(9) 22.5	0.675 cc.	6/10	0.405 cc.	Dead.

The relative potency of this series is 100 per cent as determined by comparison with ouabain as standard.

Table X

Ouabain Solution As Standard.

Dilution of Solution
1:10,000

Wt. of Frogs in grams.	Normal Dose.	Seriesof dosage.	Actual Dose.	Results
(1) 22.1	1.105 cc.	10/10	1.105 cc.	Dead.
(2) 22.6	1.130 cc.	8/10	0.904 cc.	Dead.
(3) 25.8	1.290 cc.	5/10	0.645 cc.	Alive
(4) 22.3	1.115 cc.	6/10	0.668 cc.	Dead.
(5) 30.9	1.545 cc.	5/10	0.773 cc.	Alive
(6) 21.5	1.075 cc.	4/10	0.430 cc.	Alive
(7) 22.4	1.120 cc.	5/10	0.560 cc.	Alive
(8) 25.3	1.265 cc.	6/10	0.759 cc.	Dead.

This sereis of ouabain solution is used as a standard for the tinctures under Tables VI, VII, VIII, and IX; resulting 0.00,000,03 gram per body gram weight of frog.

Chemical Assay:

In connection with the biological assay of the native Oregon foxglove, a chemical determination of the activity of the active principles was also made on the monthly samples. The method used was the colorimetric method proposed by A. Knudsen and M. Dresbach (27), as follows:

5 cc. of the tincture are placed in a 25 cc. volumetric flask and diluted with water to about 15 cc.; 2.5 cc. of neutral lead acetate solution, 10 per cent in strength, are then added, the content is mixed thoroughly and the volume is made up to 25 cc. These are mixed and allowed to stand a minute and filtered. Exactly 12.5 cc. of the filtrate are then measured into another 25 cc. volumetric flask and 1.25 cc. of a 10 per cent solution of exsiccated sodium phosphate are added. The volume is made up to 25 cc. with distilled water; the whole is mixed and then filtered. 5 cc. of the filtrate are transferred to a 10 cc. volumetric flask and at the same time 5 cc. of the standard ouabain solution containing 0.266 mg. of crystalline ouabain in 5 cc. of water, are transferred to a similar flask. To each of these flasks are added 5 cc. of alkaline picrate solution (95 cc. of 1% purified picric acid are mixed with 5 cc. of 10% hydroxide solution), mixed thoroughly and allowed to stand for 20 minutes. A color comparison is then made with a standard colorimeter, the standard set at 20 mm.

The method of calculation is as follows: The depth of the unknown in mm. divided by the reading of the standard, and multiplied by 2 times the number of mg. of drugs in the aliquot portions of the specimen tested gives the number of mg. of drugs equivalent to a cat unit as expressed by the Hatcher-Brody Method (28).

Results:

Samples tested.	Readings in mm.	Number of mg. in a cat unit.
(1). Nov. collections:		
a. First year plants.	38 mm.	190 mg.
b. Second year plants.	42 mm.	210 mg.
(2). Dec. collections:		
a. First year plants.	31 mm.	155 mg.
(3). April collection:		
a. First year plants.	33.2 mm.	166 mg.
(4). June collections:		
a. First year plants.	24 mm.	120 mg.
b. Second year plants.	26.3 mm.	131.5 mg.
(5). July collections:		
a. First year plants.	20.6 mm.	103 mg.
b. Second year plants.	22.8 mm.	110.4 mg.

In experimenting with this colorimetric method proposed by Knudsen and Dresbach, the author made several attempt but was not able to get any close results. A great percentage of error is noticed, which is due to the disability of comparing the intensity of the unknown tincture with the standard solution. The standard ouabain solution had a dark yellowish color but the tincture remains a light greenish in color, even after the addition of the neutral lead acet-

ate solution and the alkaline picrate solution. The greenish tint in the tincture is no doubt due to the pigment (chlorophyll) of the digitalis leaves. The volume of neutral lead acetate solution called for in the method was either insufficient or that the Oregon digitalis has a greater content of the chlorophyll than the ones which Knudsen and Dresbach experiment with. Since the author was not able to compare the intensity of the color of the two solutions, he made his reading by measuring the tint of the color of the two solutions.

Summary and Conclusions:

(1) The potency of the wild native Oregon Digitalis purpurea L. was studied through monthly collections of the first and second year plants whenever there was possibility of getting a collection of the leaves.

(2) Tinctures were made from these monthly collection to conform to the formula and directions as given in the United State Pharmacopoeia X.

(3) The prepared tinctures were biologically assayed by the Official "One-hour" Frog Method in order to determine their potency.

(4) Crystalline ouabain solutions were used as a standard.

(5) The results of the experimentation are given in percentage strength of the solution.

(6) In view of the fact that the entire Pacific Coast, esp-

especially the Northwest Area, met with unusually long cold weather this year 1932-1933, the results obatined from the monthly collections showed very high percentage in strength.

(7) Chemical determination of the potency of Oregon foxglove were also made on the monthly collected samples.

(8) The chemcial method used was the Colorimetric Method proposed by A. Knudsen and M. Dresbach.

(9) No close results were obtain due to the disability of measuring the color intensity of the tincture with that of the standard ouabain solution in the colorimeter.

(10) Through experimentations the following conclusions were derived by the author:

(11) That the first year leaves of the December collection equal in potency to the first year leaves of the April collection.

(12) That there is very little difference in potency between the first and second year plants.

(13) That this slight difference is lost when both the first and second year plants have reached their maturity; that is, when the plant is in full bloom.

(14) That the author found only a slight difference in the susceptibility of frogs to cardiac stimulants throughout the year as seen through their reactions toward

ouabain, which was used as a standard.

(15) That when the digitalis plants were fully mature, they were equal in potency to that which is called for in t the United State Pharmacopoeia X. and by the International Protocol.

(16) That the author believes that with the usual Oregon weather (exceptional cold weather during 1932-1933) the potency of Oregon foxglove would go above than that called for in the United State Pharmacopoeia X.

(17) That with the soils found in the western coast of Oregon, especially in Lincoln County, digitalis could be cultivated for commercial purposes is the problem of cost of labor could be taken care of.

(18) That the difficulty in the colorimetric method is due to the inability to match the color of the tincture with that of the standard ouabain solution .

(19) That the cause of this difficulty is more or less due to the disability of the neutral lead acetate solution to decolorized all the green pigments of the digitalis leaves, or that the Oregon digitalis has a greater content of chlorophyll.

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