THE SOILLESS CULTURE OF DIGITALIS

BY

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Introduction

The total plant drug needs of the United States, as compared with other commercial crops, are very small. Concerning Digitalis, it has been stated that the wild supply would be sufficient for our drug needs. This supply, however, is subject to a wide variation in active principles contained therein. This variance reaches such low levels in some instances that the cultivated product is preferred.[1]

The United States produces only a limited quantity of all the important botanicals used in this country. It is true that we produce many botanicals and some of these in large quantities, but many of the most important and widely used drugs are derived from foreign sources.[2] It has been proven in the past that our dependence upon foreign sources can lead to serious shortages in time of world crisis. In both 1915 and 1941 the country was faced with such conditions, and in both instances the study of domestic cultivation was stimulated.

Pottog observed wide variations between individual plants and suggested the possibility of improved yield by selection.[3]

Newcomb suggested a study of soil, climatic conditions, and time of harvest to increase yields.[4]

Belenkii stated that the effect obtained from any crude drug was a net result of the toxic and therapeutic principles present. In some instances toxicities were increased by the cultivation of the plant. It was therefore postulated that therapeutic values could be increased and toxicities decreased by carefully controlling plant growth.[5]

Although the trend today is away from plant drugs and towards the use of their chemical entities, the latter being sometimes synthesized, there are some plant products that probably never will be replaced. Digitalis is one of these. The large glycosidic molecules of the active principles defy synthesis. Digitalis is, without a doubt, the most useful remedy in the treatment of heart failure. In some forms of cardiac disease, it is nearly a specific.[6]

The soilless growth of plants presents itself as an excellent means of controlling the mineral intake of the plant to a minute degree. This method may be modified in a number of ways that would possibly be adaptable to the experimental growth of medicinal plants.

For these reasons, it seemed desirable to determine if Digitalis would grow under nutrient solution environment, and if so, what other external environment was most beneficial or detrimental to its growth.

History, Lore, and Description

Digitalis is said to have been introduced into Europe by Irish Monks, but the first tangible evidence of its use is found in the fact that it was used in certain preparations by the mysterious Welsh "physicians" of Myddrai. There lived in Myddrai a certain family which handed down herbal recipes from generation to generation. They were first written down in the eleventh century under the patronage of the Lord of the Manor Rhys Gryg.[7]

The next chronological mention of the drug occurred in 1250 A. D., when it was listed as an external remedy,[8] the value of which it is difficult to perceive today.

The origin of the common name fox glove can be traced to the twelfth century when it was called fosglove, derived from Saxon fosglew, or fox music. This was an illusion to the ancient musical instrument consisting of bells hung on an arched support.[9]

Fuchsius, a Bavarian physician, first demonstrated its use in what might be called rational medicine. He referred to its use in the treatment of dropsy in the sixteenth century. Fuchsius, in his "Plantarum Omnium Nomenclaturae" of 1541 first latinizes the name of the plant. The latinized "Digitalis" was derived from fingerhut (fingerstall).[10] Thus both of the common names in use today have been derived from the appearance of the flower.

The common variety in medicine has a purple flower, hence the species name purpurea.

In the sixteenth century Digitalis passed into the Herbals and in 1597 was mentioned by Gerarde (John Gerard, the English surgeon and botanist). In his "Herball or general historie of plantes," he stated ".... it doth cut and consume the thicke thoughness of grosse and slime flegme and naughtie humours."

In 1640, Parkinson, apothecary to King James I, observed its value in "... extenuating tough flegme or viscous humours troubling the chest, ... there are few physicians use it and it is in a manner wholly neglected."

Ten years later Digitalis appeared in the London Pharmacopoeia but fell into misuse because of confusion between tuberculosis, for which Digitalis is quite ineffective, and dropsy of the chest, which it relieves rapidly.

Its reintroduction into medicine can be accredited to the English physician, William Withering. In 1775 he heard of an old woman of Shropshire, England who was using a certain decoction for the successful treatment of dropsy. He ascertained during the following year that the active herb of this mixture was Digitalis, and in 1785 set forth his principles in a monograph entitled "An Account of the Foxglove and some of its Medicinal Uses With Practical Remarks Regarding Dropsy and Other Diseases." Withering heralded the

modern use of Digitalis with the concluding three "inferences": Digitalis may be used with advantage in every species of dropsy, except the encysted; that it may be made subservient to the cure of diseases unconnected with dropsy; that it has a power over the motion of the heart to a degree yet unobserved in any other medicine, and that this power may be converted to salutary ends."[11]

Extensive use of the drug for criminal purposes was revealed a number of years ago in connection with a widespread insurance swindle. During the slump of 1931, certain firms of lawyers persuaded holders of disability policies to submit fraudulent claims. Doctors were involved, their part being to teach prospective claimants to simulate heart attacks. Digitalis was administered to alter the heart rhythm, deceiving the reputable specialists.[12]

The position which Digitalis occupies in the plant kingdom may be represented as follows:

Division - Spermatophyta

Subdivision - Angiospermae

Class - Dicotyledonae

Family - Scrophulariaceae

Genus - Digitalis

The appearance and common occurrence of Digitalis has given rise to many common names besides foxglove. A few

of these are:[13]

Bloody Finger	Finger Flower
Dead Man's Bell	Flop Dock
Dead Men's Bellows	Mammy-neetcap
Dog fingers	Purple Finger
Dragon's Mouth	Snapdragon
Fairy Cap	Witch's Thimble
Fairy Fingers	

The plant is a biennial herb but frequently grows into the third year. The underground portion of the plant consists of a fibrous root system which, during the first year, sends forth a rosette of long stalked, ovate to ovate-lanceolate, radical leaves. During the second summer, a single, erect, downy and leafy stem arises from the center of a leaf rosette to the height of 1 to 1.5 meters and terminates in an elongated raceme of large, purple, tubular-campanulate (bell-shaped) flowers. The lower leaves are ovate to ovate-lanceolate, pointed, up to twelve inches in length and three in breadth, and possess winged petioles; the upper are alternate, sparse, and lanceolate; both are irregularly crenate to dentate and have wrinkled pubescent surfaces, of which the upper is a fine deep green, the under paler and more downy. The flowers are numerous, and attached to the upper part of the stem by short pedicels, in such a manner as generally to hang down upon one side. At the base

of each pedicel is a bract, which is sessile; ovate, and pointed. The calyx is divided into five segments, of which the uppermost is narrower than the others. The corolla is gamopetalous, tubular bell-form, swelling on the lower side, irregularly divided at the margin into short obtuse lobes, and in shape and size not unlike the end of a finger of a glove. The mouth of the flower is guarded by long soft hairs. Externally it is generally a bright purple; internally, it is sprinkled with dark spots upon a white background. There are four didynamous stamens whose filaments are white, curved, and surmounted by large yellow anthers. The style is simple, and supports a bifid stigma. The seeds are numerous, very small, grayish-brown, and contained in a pyramidal two-celled capsule.[14]

Digitalis purpurea L. has been official in most pharmacopoeias since antiquity. It has been official in the Pharmacopoeia of the United States (U. S. P.) since its first publication in 1820. The part most used has been the leaves.

Digitalis lanata Ehrh. is the official source of Digoxin U. S. P. and Lanatoside C U. S. P. This species, as will be mentioned, is preferred in all instances by some authorities. D. lanata is a perennial or biennial herb. The most striking difference from D. purpurea being in the flower color, which is creamy yellow or light purple. It

is characterized by having light grayish-green, nearly glabrous (smooth), decurrent (base extending downward), sessile, olanceolate to lanceolate leaves. The margin is entire or slightly toothed. The flowers are small.[15]

A number of other varieties and species exist in cultivation and commerce:[16]

D. purpurea L. var. gloxinoeflora Hort. has longer racemes with larger and more spotted flowers.

D. purpurea var. alba Hort. possesses white flowers.

D. purpurea var. campanulata Hort. is a large form whose upper flowers are united into a large bell-shaped bloom.

D. purpurea var. monstrosa Hort. is a double peloric form. (Increased number of regularities occurring in place of normal irregularities.)

D. thapsi L. (Spanish Digitalis) has yellowish hairs and is also less decurrent into the petiole.

D. mariana Boiss. has hoary, white, dense hair coating the stalked leaves, especially when young, and has small, scale-like bracts.

D. orientalis Lamarck is a perennial herb native to Asia Minor, characterized by slenderness and red striped, cream colored flowers.

D. lutea Linne is native to southern Europe and is characterized by smooth leaves.

D. sibirica Lindl is said to be more potent.

D. ambigua Murr. is indigenous to Europe and West Asia and is characterized by leaves with a serrated margin.

All of the species of the genus Digitalis seem to have similar effects upon the system.

Chief adulterants for Digitalis have been the leaves of unofficial species.

Fresh digitalis leaves are without odor but acquire a characteristic odor when dried and rubbed in the hand. The color of the dried leaf is a dull green, modified by the whitish down upon the under surface; that of the powder is dark green.

The seeds contain more of the active principle than the leaves, are less likely to suffer in drying and keep better but are, nevertheless, little used, owing to their higher cost.[17]

Habitat

Digitalis (purpurea and lanata) is indigenous to Southern and Central Europe but is now cultivated widely in the Americas, particularly in Washington, Oregon, Michigan and New York.[18]

Formerly the great bulk of our supplies of digitalis used in the United States came from Europe, especially Germany; in 1934 there was imported over 65,336 pounds. In 1940 only 3,038 pounds were received from Europe. Sufficient digitalis was grown in America during 1942 to 1946 and sufficient gathered from naturalized plants to supply domestic needs.[19]

Cultivation

Digitalis has been cultivated for two purposes. The first and most important is for the production of the dried leaves for medicinal purposes. The second is for decoration. The flowers themselves are not particularly pretty but the whole plant has a handsome appearance and the tall spikes are a welcome addition to any floral garden. One of the most common uses along this line is as a border flower.

The method of handling digitalis as a crop is quite similar to that of early cauliflower.[20] Viable seed may be obtained from certain reputable dealers or government sources. The seed is started in early March[21] in a rich light loam, high in humus. This may be done either in clay pots covered with window glass to retain moisture or in flats, using a propagator to maintain temperature and humidity. The seeds must be sown shallow due to their small size (10,200 per gram).[22] This may be done by spreading the seed on a smooth soil surface, then covering it with a light layer of the same soil containing 40% sand. The approximate sprouting time for D. purpurea is 9-13 days, while that of D. lanata is 13-15 days. In about three weeks the plants have one or two leaf pairs and should be transplanted to larger flats with greater spacing allowed. They are then allowed to grow until late May or early June, the time of transplanting. The plants should be hardened off in cold frames about five days previous

to transplanting. It has been shown[23] that the best time for transplanting in New Hampshire climate is around June 1. Pottog states that some varieties responded best to fall planting.[24] Transplanting should be done during a cloudy, wet spell.[25] The plants should not be watered just previous to transplanting, but watered heavily after the procedure. Care should be taken because the crown leaves are very tender and easily harmed by soil and water. The plants should then be shaded from sun for one to two days.[26] Watering is essential for normal foliage growth.

Apparently Digitalis likes wild conditions, since it grows very well in soil that has not been cultivated for a number of years. It will grow rapidly with apparently little fertilization, provided it has enough water. It also grows well in sandy or gravelly soil and vegetative growth is much increased in humid conditions. Boshart, working with purpurea and lanata showed that artificial fertilizers were beneficial. Fertilizing with ammonium sulfate gave a great increase in harvest. Phosphate and potassium gave a smaller increase while horse manure proved unfavorable.[27] Contrary to these results were those obtained by Hepler, who found in 1940 that Digitalis did not respond to the use of manure or commercial fertilizers, but these tests were done on new soil.[28] Lefrancois concurred with Boshart, in showing that potassium and phosphate were favorable to weight of harvest

but obtained no results with magnesium sulfate.[29] This shows that the favorable results obtained by Boshart with ammonium sulfate were due to the presence of ammonium ion rather than the sulfate. Parisi stated that plants of Digitalis purpurea from soil treated with fertilizers containing nitric nitrogen, phosphorous pentoxide, calcium and magnesium gave the most active extracts.[30] Sanna indicates that Digitalis prefers siliceous soil, and tolerates up to 5-6% Calcite.[31] Even in Calcite soils in which the proportion of calcium is very small, normal digitalis decreases in glucoside content. Mayer also reported that calcium is unfavorable to Digitalis but otherwise requires a soil rich in nutrient minerals, especially potassium.[32]

An interesting point regarding growth has been shown by McCrea.[33] A section of a greenhouse was built of special glass which transmitted a certain portion of the ultra-violet spectrum. Digitalis plants in the two leaved stage were placed under the special glass, with adequate controls kept under ordinary glass. Those plants grown under the special glass continually held the growth advantage, the leaves appearing sooner and general plant growth being better. After transfer to outdoor conditions the growth advantage became not so striking but carried over to the first cutting, at which time those plants grown under special glass showed an average increase in physiological activity of 19%.

At the time of the second cutting the visible growth advantage was gone but activity of those started under special glass was 34% greater. These results carried into the third cutting.[34] In the second year of growth the amounts of physiologically active principles were less, showing that first year plants are most active, but the same relative differences between the two groups of plants existed.[35]

The crop grows most readily during the cool, short, fall days of September and October, until the time of freezing. Freezing does not affect the content of active principles.[36]

Newcomb states that early cutting hastens vegetative growth in later stages.[37]

Immersing the plants in an atmosphere of carbon dioxide for several weeks appeared to stimulate vegetative growth, and carbon dioxide treatment for one day increased pharmacological values.[38]

The difficulty in the culture of Digitalis in the Great Lakes region is their high mortality in winter.[39] This may be overcome by a light covering of straw or burlap. The covering must be light to avoid smothering and drainage must be good to avoid root rot.[40] The covering is to prevent heaving, to which the plant is susceptible because of the shallow root system.[41]

Second or third year plants normally give nice flowers.[42]

The occurrence of a large, bell- or cup-shaped flower at the apex of the inflorescence is an abnormality which has frequently been recorded.[43]

Arthur reports that greenhouse plants which fail to flower may be made to flower by four months of night temperatures at 41° F. This flowering response in plants brought about by low temperature is called thermoperiodism.[44]

Hecht stated that the climate has the greatest influence on strength of active principles, while the greatest effect of soil is to increase yield.[45]

Digitalis is host to several insect pests. Sievers states that the Red Spider does some damage to D. purpurea.[46] The Foxglove Aphid (Myzus pseudosolani) uses D. purpurea as a primary food source during the first, second, and last two generations of its life cycle. Damage is inconspicuous to casual examination unless laying is quite heavy.[47] D. purpurea is also a host for Tylenchus dipsaci, the bulb or stem nema.[48]

Collection is made of both first and second year plants in late summer. This should take place in late afternoon because the glycosides are hydrolyzed at night.[49] A good yield is around 3/4 ton of dried leaf per acre.[50] The recommended method of drying involves the use of large drying ovens, in which the temperature can be most accurately controlled. Treatment at 100° C. for three days, eight hours

per day, reduces moisture to 4%, destroys the ferments, fixes the green color, and does not injure the active principles. Other desirable procedures involve uniform collection by experienced persons and storage under dry conditions.[51]

Boshart states that formation of glucosides is in direct proportion to the assimilatory action of leaves, while alkaloidal manufacture is not directly related to assimilation, but to later transformations in the plant.[52] Following this reasoning, it would be true that the plants showing the most growth would be most potent in active principles.

Heritable differences both in activity per unit weight and in yield of leaf exist between strains within Digitalis purpurea and Digitalis lanata. Strains obtained from commercial cources were not superior in either respect to strains from the wild and from botanical gardens. The latter showed, in fact, consistently higher degrees of activity than the former. It is concluded that the breeding of improved strains has not been undertaken in the past but would be successful if on adequate scale. The isolation of superior strains from the wild is worthy of attention in the meantime.[53]

Constituents

Glycosides, the most important constituent of Digitalis purpurea, have been isolated in concentrations up to 1% of the dried leaves.[54] Glycosides may be related to the most important type of plant constituent, the carbohydrates, or more specifically, the sugars. Apart from the existence of sugars as saccharides, they may also be found in a large class of substances in nature known as the glycosides, containing sugars in combination with non-sugars. "One of the most important methods of investigation of such substances consists in their hydrolytic cleavage into the two major constituents, the sugars, called the glycone, and the non-sugars, termed the aglycone."[55]

Glycosides occur throughout the plant kingdom in all tissues. Certain glycosides are characteristic of certain plants. A good example of this is Digitalis, in which cardiac glycosides are characteristic. The aglycone portions of the molecule contain the therapeutically active principles, e.g. the analgesic properties of gaultherin are inherent in the salicylic radical of the aglycone and the cardiac properties of digitoxin reside in digitoxigenin, as contrasted with digitoxose, the glycone. Ferments (enzymes specific for the particular glycosides) occasionally accompany the glycosides within the plant but are separated by one or more plant tissues. Required then, for hydrolysis,

are excess moisture and possible bruising of the tissues.

The teleology of the glycosides has never definitely been decided, many theories having been promulgated. The cardiac glycosides in particular have been subjected to the following suppositions:

1. By virtue of their extreme toxicity and bitterness they act as protectors from vermin and parasites, and exist in plant tissues for that purpose.
2. They are waste products of metabolism. The odd sugars residing in these glycosides could possibly be the results of misguided synthesis reactions, supporting this theory.
3. They serve as solubilizers of the sparingly soluble steroid lactones.
4. They are storage forms for carbohydrates.

The work done by Withering instigated centuries of investigations of the leaf of Digitalis purpurea. All the cardiac glycosides that have been investigated in detail are hydroxylactones of sterolhydrocarbons in which one hydroxyl group is connected with a sugar molecule or a chain of several sugars. The rings A and B of the sterol portion may be either cis or trans. The hydroxyl group on carbon atom # 3 may be either cis or trans to C_{18} but is generally trans. The principal aglycons have been shown to differ

from each other chiefly in the number and positions of the hydroxyl groups and in the stereochemical nature of the ring system as shown in Table I.[56] Though the pharmacological activity of the glycoside resides in the aglycone portion of the molecule, the sugars when combined with the aglycones increase both the potency and toxicity of the active principle. In addition, the sugars affect certain physical properties of the chemical combination, such as water solubility, and diffusion and persistence of the therapeutic action.[57]

Until the undertakings of Stoll and co-workers, three glycosides had been isolated in a chemically pure state, digitoxin, gitoxin, and gitalin. Their formulas and hydrolysis products are shown in the table set forth by Stoll. (Table III)

Digitoxin in pure form is sparingly soluble in water and is a well-defined, colorless, odorless, crystalline, bitter substance. It is more or less soluble in alcohol and colored green with hydrochloric acid. It may be identified by Keller's reaction, which consists in dissolving it in glacial acetic acid, adding a drop of ferric chloride solution, and then, gently, adding sulfuric acid to form a layer below the acetic acid. A brownish green band is first formed, after which the acetic acid layer becomes greenish blue and then indigo blue, while the sul-

furic acid becomes brownish red. Digitoxin is the most toxic of the substances obtained from the leaves, and is accumulative in action. Depending on the process of extraction and the degree of purification, commercial digitoxin contains more or less gitoxin and possible small amounts of other digitalis glycosides.[58]

Gitoxin occurs as white needles, very slightly soluble in water, alcohol or chloroform.[59]

Gitalin occurs as white rosettes. It is soluble in alcohol, chloroform and acetone.

The glycosides existing in the seeds of D. purpurea differ considerably. One of these is Digitalinum verum. Severe conditions are required for its hydrolysis, as in comparison with the other glycosides mentioned above. Jacobs explains varying conditions required for hydrolysis of glycosides.[60] He noticed that only those glycosides containing an alpha deoxy sugar are subject to easy hydrolysis. Thus digitalinum verum, containing glucose and digitalose (non-alpha deoxy sugars) requires drastic conditions for hydrolysis as contrasted with the three above mentioned glycosides of the leaves.

A number of saponins occur in the leaves and seeds of D. purpurea. These are digitonin, gitonin and tigonin, and are without cardiac action.

Infusions of Digitalis contain up to 5 gamma nicotinic acid per 100 cc.[61].

Digitalis lanata was subjected to investigation in more recent years, as compared with D. purpurea. Smith succeeded in isolating the glycosides gitoxin and digoxin from this source.[62] The most important work with this source has been done by Stoll, who first worked on squill, using new concepts of extraction, which later became of utmost importance in work on the digitalis glycosides. His methods involved the following principles:

1. Use of raw material which was as fresh as possible, thus providing unchanged source.
2. Mild treatment during the grinding, extracting and purifying operations. Enzymatic action was avoided and degradation by acids, bases, light, oxygen and expecially heat was avoided.

Impetus for the work done by Stoll was the desire to obtain the natural, native glucosides. He noted that physiological action was inherent in the aglucone portion but was greatly increased by the presence of the lactone ring and the solubilizing activities of one or more sugar groups attached to the sterol nucleus through glycosidic linkage. Improper drying or storage so rapidly decomposed the glycosides that it was not realized until approximately 1930 that the known glycosides were but derived products from glycosides of higher carbohydrate content. The aim was "to isolate the genuine digitalis glycosides in a chemically pure state, and then to apply these, not singly,

but as mixtures of the most important representatives in suitable proportions in order to obtain a product of constant activity." Stoll obtained, by precipitation with neutral salts, a mixture of the glycosides and their inactivated enzymes. Further separation yielded the glycosides, mucilaginous in nature, existing as their tannoids. These tannoids were separated from the steroid fraction by virtue of their insolubility in ether. Removal of the tannin portion was accomplished with an insoluble precipitant ($Pb(OH)_2$) and repeated extraction from methanol gave a mixture which showed no change in properties . He called this substance digilanid. Stoll assumed this to be chemically pure. Hydrolysis, however, showed the presence of different aglucones. It was suspected that this was a mixture and the next steps, limited to physical methods, (namely about forty extractions with water, chloroform, and an intermediate layer) yielded the individual glycosides in their native proportions:

Digilanid A	46%
Digilanid B	17%
Digilanid C	37%
Purpurea glycoside A	
Purpurea glycoside B	

The schematic summary of this extraction set forth by Stoll may be seen in the reference.

Equations for the hydrolysis of the known existing glycosides are shown in Table II.

Due to greater content of glycosides, greater number of glycosides and preferred analytical processes, Stoll advocated the digilanid mixture in preference to purpurea products for both manufacture and therapy.

A new glycoside called diginin was isolated in 1936 by Karrer[63]. This was shown to differ from the other digitalis glycosides in the lactone portion[64]. It is decomposed by very dilute mineral acids to crystalline diginigenin and diginose. Diginose was shown to be a two desoxy sugar, ($C_7H_{14}O_4$), containing a methoxy group. It was further shown to be isomeric with cymarose, the methoxy position differing.[65] Diginigenin ($C_{21}H_{28}O_4$) contains a reactive aldehyde group and a hydroxyl group.

TABLE I

Aglycon Structure of the Cardiac Glycosides

Glycoside	Aglycon	Position OH groups	R	Rings A/B	C_3OH/R
Digitoxin	Digitoxigenin	3,14	CH_3	Cis	Trans
Thevetin	Thevetigen	3,14	CH_3	Cis	Cis
Uzarin	Uzarigenin	3,14	CH_3	Trans	Cis
Digoxin	Digoxigenin	3,12,14	CH_3	Cis	Trans
Gitoxin	Gitosigenin	3,14,16	CH_3	Cis	Trans
Periplocymarin	Periplogenin	3,5,14	CH_3	Cis	Trans
Sarmentocymarin	Sarmentogenin	3,11,14	CH_3	Cis	Trans
k-Strophanthidin	Strophanthidin	3,5, 14	CHO	Cis	Trans
Cymarin	"	same			
Convallatoxin	Convallatoxigenin	3,5,8,14	CH_3	-	-

R is radical located on Carbon No. 18.

Table II

Digitalis Glycosides

Arranged in the order of their aglucones and their sugar content

Digilanid A	Digitoxigenin	3 Digitoxose + Glucose + Acetic acid
Deacetyl digilanid A (Purpurea glycoside A)		3 Digitoxose + Glucose
Acetyl digitoxin (α & β)		3 Digitoxose + Acetic acid
Digitoxin		3 Digitoxose
Digilanid B	Gitoxigenin	3 Digitoxose + Glucose + Acetic acid
Deacetyl digilanid B (Purpurea glycoside B)		3 Digitoxose + Glucose
Acetyl gitoxin (α & β)		3 Digitoxose + Acetic acid
Gitoxin		3 Digitoxose
Gitalin	Gitoxigeninhydrate	2 Digitoxose
Digitalinum verum	Dianhydrogitoxigenin	Digitalose + Glucose
Digilanid C	Digoxigenin	3 Digitoxose + Glucose + Acetic acid
Deacetyl digilanid C		3 Digitoxose + Glucose
Acetyl digoxin (α & β)		3 Digitoxose + Acetic acid
Digoxin		3 Digitoxose

Processing and Preparations

Following the drying of Digitalis leaves, they are put to use in one of the two following procedures:

1. The use of the leaf as such for the preparation of galenicals.
2. Extraction of the glycosides.

Hepler states that the greatest and most serious variable in Digitalis production is the drying process. He makes the following recommendations:[66]

1. Collection late in the first year of growth, due to the increased growth rate at this time of year.
2. Stripping of the leaves from the midrib and petiole, due to their low glycosidal content and resistance to drying.
3. Quick drying at temperatures ranging from 150 to 160° F. (65 to 71° C.).

The official method of extraction for Digitalis Tincture U.S.P. XIII and Digitalis Extract U.S.P. XIII involves a menstruum of alcohol and water. Chase[67] showed that products prepared with isopropyl alcohol as the menstruum compared favorably with those prepared by the official methods.

Solid Digitalis preparations are the most stable, while the liquid preparations show a decrease in pharmacological activity. Digitalis Tincture exhibits this phenomenon, and

contains many inactive ingredients which hasten decomposition, and should be barred from the Pharmacopoeia.[68] Copper and Zinc in correct quantity tend to stabilize this preparation.[69]

Exhaustive methyl alcohol extraction isolates the glycosides. Chemical and physical purification and isolation may then follow, to give the various glycosidic preparations obtainable.[70]

The Digitalis preparations in official and semi-official literature[71, 72, 73] are as follows:

Digitalis U.S.P. - The dried leaf of D. purpurea

Powdered Digitalis U.S.P. - The powdered leaf of D. purpurea.

Digitalis Capsules U.S.P. - Contain Powdered Digitalis.

Digitalis Infusion N.F. - An infusion of Powdered Digitalis.

Digitalis Injection U.S.P. - Sterile, aqueous solution of a mixture of glycosides or therapeutically desirable and cardioactive constituents of Digitalis.

Digitalis Tablets USP - Contain Powdered Digitalis.

Digitalis Tincture U.S.P. - Prepared by Process P.

Digitoxin USP - Pure digitoxin or a mixture of cardioactive glycosides obtained from D. purpurea.

Digitoxin Injection U.S.P. - A sterile solution of Digitoxin.

Digitoxin Tablets U.S.P.

Digoxin U.S.P. - A glycoside obtained from D. lanata.

Digoxin Injection U.S.P. - A sterile solution of Digoxin in 70% alcohol.

Digoxin Tablets U.S.P.

Lanatoside C. U.S.P. - A glycoside obtained from the leaves of D. lanata.

Lanatoside C. Injection U.S.P. - A sterile isoalcoholic solution of Lanatoside C.

Lanatoside C. Tablets U.S.P.

Digalen N.N.R. (Hoffman - LaRoche) - The cardioactive principles of Digitalis.

Digifolin N.N.R. (Ciba) - A preparation containing the therapeutically desirable constituents of digitalis leaf.

Digilanid N.N.R. (Sandoz) - A mixture of Lanatosides A,B and C in proportions in which they occur in the crude drug.

Digitan N.N.R. (Merck) Natural extracts, free from digitonins.

Digitol N.N.R. (Sharp & Dohme) - A fat-free Digitalis tincture.

Gitalin N.N.R. (Rare Chemicals Inc.) - A glycoside of D. purpurea

Unitage and Assay

"The potency of Digitalis is such that, when assayed as directed, 0.1 Gm. shall be equivalent to not less than 1.0 U.S.P. Digitalis Unit." A variance of ± 20% is allowed inall Digitalis products in the U.S.P. "One United States Pharmacopoeial Digitalis Unit represents the potency of 0.1 Gm. of the U.S.P. Digitalis Reference Standard."[74] This U.S.P. Unit is identical with the International Unit, adopted in 1928 by the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations.[75]

The U.S.P. contains two assays relative to Digitalis. One is the Cat Method of Hatcher and Brody, the other is the Colorimetric Digitoxin Control. The first method of the Colorimetric Control involves the alkaline picrate color reaction of Knudson.[76] The second method involves a ferric chloride color reaction. In these assays, values are obtained by comparison of results between the sample and U.S.P. Reference Standard Digitalis.

The bioassay of Digitalis is made necessary by the fact that pure principles are not generally available and most of the practice is confined to the use of the crude material or mixtures of the glycosides. There are no satisfactory chemical methods for their estimation. Numerous methods have been suggested for the assay of

Digitalis, embracing a wide variety of animals and techniques. One favorite objection to existing animal assays is that the end point is death, thus the assay is a measure of the toxicity, rather than the therapeutic activity, but no significant proof of variance between ratio of therapeutic and toxic activities has been given.[77]

The following are methods of determination of relative potency of Digitalis preparations[78]:

Goldfish Method

Daphnia Method[79] - The heart beat of the daphnia is first depressed by the use of yohimbino[80], an alkaloid obtained from the bark of Corynanthe Yohimbischum, then graded doses of the Digitalis preparations are used to antagonize this depression. The approximate strength of the preparation may be computed from the minimal amount required to counteract the depressant action. Comparison is made with a reference standard.

Pigeon Emesis Method

Cat Method

One hour Frog Method

Twelve hour Frog Method

Eighteen hour Frog Method

Guinea Pig Method

Various other miscellaneous methods involving heart perfusion, use of isolated strips of heart muscle, humans and dogs.

The real problem seems to be that when two preparations of digitalis are compared by different methods or in different animals, different answers are obtained. The question arises, which of the methods give results valid for man?

The two main methods are the Cat and Frog method. Unfortunately, neither overcome all of the obstacles, due to differences in absorption methods of the two animals. Experiments were made which showed that therapeutic activity runs parallel to T wave changes in the human electrocardiogram, thus representing a possible human assay.

Another solution is the use of purified glycosides, whose activities can be expressed in terms of chemical entities. These are becoming increasingly available.[81]

A review of the three most important methods of bioassays is given in Table III.

There are possibilities of accurate colorimetric assays. M-dinitro benzene reacts with digitoxin to give a brilliant blue color but the color fades. A quantitative determination can be accomplished by the measurement of the

extinction coefficients.[82]

Certain color reactions of identification can be adopted to quantitative means. These reactions involve Digitalis and Digitoxin and can be applied to either dry substances or official solutions.

1. A few tenths of a milligram of digitalis in a porcelain evaporating dish with one ml. of digitalis reagent (0.1 Gm. dimethyl-aminobenzaldehyde dissolved in 20 ml. Ethyl Alcohol and four drops conc. H_2SO_4.) is evaporated on a boiling water bath. As the liquid evaporates, aseries of red zones appears. When the evaporation is complete, 20 drops of water give an intense eosin red color, stable and miscible with water. Glycerine interferes with this reaction and must be removed if present in official solutions. Digitonin gives the test but can be distinguished from Digitalis by treating with a mixture of one drop of aqueous bromine and 20 ml. Conc. H_2SO_4. This gives a cherry red with Digitalis but none with digitonin.
2. Digitoxin and vanillin reagent (30 ml. pure vanillin in 100 ml. iron free HCl) give an

intense indigo blue. Pure crystals can be detected by heating a few tenths of a milligram dissolved in two drops of glacial acetic acid over a water bath with 10 ml. of vanillin reagent. First a red color appears which changes to a stable blue, the amount of digitoxin being proportional to the depth of the color.

3. In a mixture of Digitalis and Digitoxin, the red color of Digitalis' reaction is normal.[83]

Table III[84]

A Review of the Most Important Methods of Bio-Assay

Method & Animal	Standard	Time Required	Mode of Administration	End Point	Value of Unit
Frog Method (U.S.P. XII) Grass Frog	U.S.P. Reference	1 hr.	Anterior Ventral lymph sac.	MSD_{50}	.0745 Gm Ref. Pdr.
Guinea Pig Method Guinea Pig	U.S.P. Reference	½-6 hr.	Subcut. in abdominal reg.	MLD_{50}	.0745 Gm.
Cat Method U.S.P. XIII Cat	U.S.P. Reference	1-1½ hr.	I.V. in femoral vein	Cessation of heart beats.	.1 Gm.

MSD_{50} - when not more than 75% and not less than 50% of frog hearts are in systole.

MLD - Minimum Lethal Dose

General Aspects of Soilless Culture

Recent popular articles have given the impression that the various types of soilless culture represent a new discovery and a shortcut to the successful growth of plants. Such is not the case. No fantastic yields or rapid growth has been reported. The main advantages which may be accomplished are as follows:[85]

1. Plant intake can be controlled accurately.
2. The lack of soil may be overcome.
3. Fertilizer costs are abolished.
4. Labor costs are minimized, although in some cases this may be overshadowed by the cost of initial outlay on a commercial crop scale.

The term soilless culture implies the growth of plants in any material other than soil.[86] This may be generally divided into three main types, water, sand and gravel culture. Of these, the gravel culture method is almost exclusively adapted to large scale crop production. The general function of these types of culture is to replace the functions of the soil, i.e. mineral source, moisture source and plant support. The other essentials of plant growth such as climate and temperature remain the same, and from this fact it is obvious that a knowledge of plant

physiology and culture techniques is indispensable.

Water culture involves suspending the roots of the plant in a nutrient solution containing the essential minerals.[87] Strictly speaking, the term hydroponics indicates this method, but has been modified by usage to include all other methods. The water culture method necessitates aeration in some manner to similate soil conditions. Aeration may be accomplished by the forcing of air through the solution, by allowing an air space above the solution level, or growing some of the roots in a porous medium. For experimental purposes it is suggested that the plants be suspended in perforated corks, held in place by cotton.[88] The greatest reasons for failure are the improper manipulation of solution levels and improper plant support.

The sand culture method consists of growing the plants in a fine medium of known mineral consistency. The nutrient is applied by the slop or drip method at the surface of the medium or by subirrigation. The sand provides excellent support for the plant and the particles used are not too fine to prevent proper aeration. This method appears as the easiest for small scale operation.

History of Soilless Culture

The development of soilless culture techniques awaited the discovery of the normal functions of soil, which took place in the middle of the nineteenth century.[89] At this time it became apparent that the plant utilized elements from air, water and soil, but that soil supplied only a portion of the substances present in the plant. Following the realization of this theory, Boussignault grew plants in artificial insoluble media, watering with chemical solutions of known content. Sachs took the next step in 1860 by dispensing with the solid medium, growing the plants in liquid only.[90]

Five years later Knop formulated a successful solution which has been in common use since.

Sachs set forth a concept of great importance.[91] He stated that a wide margin may be permitted in the concentration of the salts supplied in the solution. The important point is to furnish the elements required, the plant using them as necessary.

The work done since the formulation of the original concepts has been of a modifying nature. It is stated that the individual techniques used must vary with the conditions and plants used. No universal method can therefore be

recommended and any of the methods in use today are merely applications of the general concepts stated above.[92]

Elements Required for Plant Growth

Analysis of plant materials proves the presence of carbon, hydrogen, oxygen, nitrogen, potassium, magnesium, calcium, phosphorous, sulfur, iron, manganese, boron, copper and zinc. Carbon, hydrogen and oxygen are assimilated by the plant in the form of water and carbon dioxide. Iron, manganese, boron, copper and zinc are classified as micro or trace elements. The remaining essentials are classed as macro elements.

External deficiency symptoms are listed in Table IV.

Table IV

General Key to Foliar Symptoms of Mineral Deficiencies in Plants[93]

Mineral Deficient

I. Initial Injury on Mature Foliage

A. Site of injury general

1. Necrosis of tissue

a. Stunted, light green plants; older leaves yellow green to yellow in color, followed by drying and browning in advanced stages.....Nitrogen

2. No Necrosis of tissue

a. Stunted, abnormally dark green plants usually with narrow-petiole angles; abundant reddish or purplish pigmentation; sometimes chlorosis of older leaves.....Phosphorous

B. Site of Injury localized

1. Chlorosis starts at tips and margins of older leaves, progressing between veins, followed by brown necrotic spots which usually fall out, giving

Table IV (cont.)

	Mineral Deficient
ragged appearance; leaves crinkled and curled, most noticeable in early stages	Potassium
2. Irregular chlorotic spots between veins in older leaves, followed by rapid necrosis and defoliation; die-back of twigs and small-leaved rosettes in fruit trees	Zinc
3. Chlorosis starts between veins in older leaves; leaves become yellow or almost white with veins usually remaining green; necrosis not usual	Magnesium
II. Initial Injury on Immature Foliage	
A. Site of Injury General	
1. Entire plant light green to yellowish green in color; chlorosis most pronounced in young leaves which become yellow	Sulfur
B. Site of Injury Localized	
1. Necrosis of tissue	
a. Intervenal chlorosis of young leaves; leaves become yellow or white in color, all veins	

Table IV (cont.)

	Mineral Deficient
remaining green; small, brown necrotic spots follow chlorosis.....	Manganese
b. Chlorosis generally begins at bases and margins of young leaves, followed by necrosis; leaves become distorted or in more severe deficiencies terminal buds die and turn brown or black in color; gummy or corky deposits occur in fleshy organs.....	Boron
c. Chlorosis generally begins at tips and margins of young leaves, progressing between veins, followed by necrosis; leaves become distorted or in more severe deficiencies terminal buds die and turn brown or black in color; roots characteristically short, bulbous, with necrotic upical meristems.....	Calcium

Table IV (cont.)

	Mineral Deficient
2. No necrosis of tissue	
a. Intervenal chlorosis of young leaves, veins remaining green, entire leaf including veins becomes yellow or white in color	Iron
b. Plants exhibit lack of turgor; wilting most pronounced in tops; sometimes chlorosis of young leaves	Copper

Solutions

In addition to the list of essential elements, which is obviously of first importance in making artificial culture media for growing plants, a large amount of information has been amassed on the desirable proportions and concentrations of elements, and on such physical and chemical properties of various culture solutions as acidity, alkalinity, and osmotic characteristics. A most important recent development has been recognition of aeration essentiality.[94] Obviously, no nutrient solution can act as a substitute for light and suitable temperature. External environment must be suitable for normal plant growth.

No evidence has been shown which leads to the conclusion that the plant product grown in nutrient culture is superior. With work done on tomatoes, no significant difference in mineral or vitamin content can be shown by nutrient culture.[95]

Plants have marked powers of adaptation to different nutrient conditions. If this were not so, plants would not be growing in varied soils in nature. Within certain ranges of composition and total concentration, fairly wide latitudes exist in the preparation of nutrient solutions suitable for plant growth.

After the plants begin to grow, the content of their

nutrient media changes, due to absorption of certain elements. A secondary result is that the pH of the solution may change.

To guard against such changes in solution content, they should be prepared with distilled water, in small amounts, and should be changed frequently.[96]

The variance in mineral needs of different plants may be disregarded. If an adequate supply of nutrient elements is present, many kinds of plants may be grown successfully. A general type of solution is recommended for beginning work.

The elements are present in the various solutions[97] in ion form, since they must be in complete solution and because the plant utilizes the elements in the form of their various ions.

The various tanks, supports and containers suggested by the authors are far too numerous to enumerate in detail. They vary from Mason and bell jars for experimental work to large outdoor tanks for crop production. Another factor determining the set-up is the type of plant grown.

Experimental

It is stated by Ellis and Swaney that seed germination may be accomplished either in a sand-water medium, water medium, sand-nutrient medium or in soil.[98] Consequently, several attempts at seed germination were made.

The three groups of seeds were sown in light loam contained in seed flats. The flats were of two sizes, 8" x 8" and 10" x 13". Soil depth was approximately 2½". Due to the extremely small size of Digitalis seeds, they were sown just slightly under the surface of the soil. The flats were placed in a propagator of inside measurements 2' 11" x 6' 11". Light and heat were furnished by two 40 Watt Sylvania Flourescent tubes, four feet in length. The temperature within the propagator was maintained between 30 and 35° C. by these lights. Watering was accomplished on alternate days.

Twelve day results (1/20/49 to 2/1/49):

Seed Group I (D. lanata from source A)

Five 3/8" seedlings were observed.

Seed Group II. (D. purpurea from source B)

One ¼" seedling was observed.

Seed Group III (D. lanata from source B)

Three ¼" seedlings were observed.

Twenty day results (1/20/49 to 2/9/49):

Seed Group I

Thirty ½" seedlings were observed.

Seed Group II

Five ¼" seedlings were observed.

Seed Group III

Four ¼" seedlings were observed.

Twenty six day results (1/20/49 to 2/15/49):

Seed Group I

Thirty eight 3/4" seedlings were observed.

Seed Group II

Five ½" seedlings were observed.

Seed Group III

Six ½" seedlings were observed.

The flats were left in the propagator and the plants of Group I continued to show growth. Those of Group II died. Three of the plants of Group III died, the remaining showed slow growth.

The second attempt at germination involved placing the seeds in moist cotton. The cotton was repeatedly moistened and supplied with a few drops of a nutrient solution daily. The seeds of Group I had sprouted within a week. Group II seeds failed to sprout. Group III seeds sprouted in eleven days. After attaining a height of

about 3/8", all the seedlings perished.

A third attempt at germination using nutrient solution and sand was made. The seeds of Group I were sown very lightly in silica sand, held in one liter beakers which were supplied with bottom outlets. Knop's solution was supplied by sub-irrigation. Fifty per cent germination was obtained but the seedlings perished after attaining a height of 3/8".

Thus, it was shown by the germination attempts that the natural medium of soil gave the best results. It was also shown that seeds from source A were more viable than those obtained from source B.

The transplanting of the specimens of D. lanata to nutrient solution environment was then undertaken. It was first decided to use Purdue Solution.[99]

Salt	Grams/l.
$MgSO_4$	.260
$CaH_4(PO_4)_2$	.135
KNO_3	.550
$CaSO_4$	.760
$(NH_4)_2SO_4$	.140

Attempts at preparation of this solution were unsuccessful, due to the insolubility of the calcium sulfate.

Attempts towards the preparation of Knop's solution[100] met with success. A solution of trace elements was added,

as indicated below.

Salt	Grams/l.
$Ca(NO_3)_2$	.8
KNO_3	.2
KH_2PO_4	.2
$MgSO_4$	.2
Trace Element Solution	
H_3BO_3	1.6
$MnSO_4$	1.6
$ZnSO_4$	1.6
$CuSO_4$	.3

Five drops of the trace element solution were added to each liter of the final nutrient solution.

The first attempt at transplanting to nutrient conditions involved the water culture method. The healthy specimens of D. lanata were suspended in perforated corks with cotton. The corks were placed in the holes of a desiccator plate and the plate was lowered over a one liter beaker. The beaker was filled with Knop's solution and level maintained so that the roots remained suspended in the nutrient. The beaker was painted with black enamel on the outside, to protect the solution from light. Aeration was by forced air. The plants never recovered from the transplanting and within two weeks were dead.

The specimens of D. lanata were transplanted to washed silica sand in the second attempt at growth in

nutrient conditions. The plants had a leaf length of about four inches at the time of transplanting. The sand was held in one liter beakers provided with bottom outlets. Glass wool pledgets protected the outlets and Knop's solution was supplied by sub-irrigation daily. The plants recovered from the transplanting within two weeks. Sunlight was supplemented with light from a 150 Watt unfrosted bulb suspended two feet above soil level. The plants continued to grow. At the last observation the average leaf length was twelve inches and the plants had a healthy appearance and color.

It was concluded that silica sand provides the proper root support and aeration for D. lanata but that light requirements require supplementation of sunlight when the growth is attempted indoors.

Summary

1. A brief pharmacognostical review of Digitalis is given.

2. Soil germination and sand culture attempts were successful with Digitalis lanata.

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