

## NOTES ON THE IRON-GREENING FACTOR OF DIGITALIS.\*

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The United States Pharmacopœia restricts the use of the name digitalis to the dried leaves of *Digitalis purpurea*, which is the material referred to in these notes.

The physiological effect of digitalis is both known and understood, but no such fortunate circumstance exists for its constituents; instead, the very opposite state of affairs prevails, and with scarcely any other drug of equally recognized value is there so much confusion.

For a century, digitalis has been the subject of numerous investigations. The reports of the earlier workers pertain chiefly to the active principles, which are commonly designated as glucosides, but are made confusing by an indiscriminate use of names.

The reason for variation in the several proposed educts has long been known; Maisch summed it up in the words—"The behavior to solvents is more or less altered by the presence of other principles."

For a time, the hopelessness of separating the active substances cast some doubt on the future usefulness of the drug, but the advent of physiological assay methods has not only saved digitalis from threatening discard but actually given it a definite value; and the United States Pharmacopœia now requires the drug and its preparations to be standardized by means of such methods.

It was natural that attention should first be given to the active ingredients; but, though much effort has been expended on the separation of these principles, comparatively little regard has been paid to the nature of the inactive constituents. Therefore, while the works of reference abound in descriptions of the active principles, but casual consideration has been given to others.

Some later workers have devoted their efforts to the production of preparations of digitalis which, while representing the activity of the drug, should contain a minimum of inactive matter. Such products are especially desirable for hypodermic use. It is evident that the improvement of this class of preparations depends largely upon a knowledge of the inactive constituents. The slight importance accorded these principles is shown by the mere listing of their names; for instance, one author after much explanation of active constituents says, "other usual constituents of plants, as tannic acid, volatile oil, coloring matter, starch, sugar, gum, salts."

Allen states "the other constituents of digitalis are not characteristic. They include chlorophyl, mucilage, albuminous matter, various salts and inosite." Besides these inactive substances, other authors mention "extractive, resin, pectin, volatile oil, fixed oil, vegetable acids (both fixed and volatile), lignin and mineral matter." Two coloring principles, besides the chlorophyl so evidently present, are described as "red" and "yellow;" the former as analogous to extractive, the latter as "resembling chrysophan, a glucoside found in rhubarb." An "oxydase ferment" ascribed to the recently dried leaves completes the list of plant principles for digitalis.

It will be noted that the presence of vegetable acids has been commonly

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stated, and they have been repeatedly separated as crystalline substances, although vaguely described and named.

In 1834, W. J. Wilding (*Amer. Jour. Pharm.*) concluded from experiments upon the leaves that they contain gallic acid, mucilage and reddish brown coloring matter, soluble in water, insoluble in alcohol or ether. He found that "digitalis imparts its peculiar properties to cold or hot alcohol, and to boiling water; the decoction or infusion reddens litmus paper, and is precipitated by salts of iron, of a black color, etc." "The nitric, hydrochloric and sulphuric acids also produce precipitates." "Alcohol added to the decoction renders it of almost jelly-like consistency, probably owing to the mucilage it contains."

These remarks of Wilding appear to be the first recorded mention of the behavior of the constituents of digitalis toward iron compounds. But Wilding only inferred the presence of gallic acid from this reaction, he did not isolate it. That this behavior toward ferric salts was or was not due to a tannin seems to have had little import until Githens published his "Studies on the Pharmacology of Digitalis Bodies" in the *JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION* for November 1920. He there states that his efforts were purposely and primarily concerning the active constituents; his interest in other constituents was only that involved in separating the active from the inactive matter.

In treating of these inactive components, he said that "in addition to the active glucosides, the leaf contains a very large amount of tannin, constituting, in water and dilute alcohol extracts, the largest part of the solids, and amounting to as much as 16 to 20 per cent. of the weight of the dried leaf." "The tannin is of great importance in helping to cause the bad taste and nauseating properties of the crude drug." "Besides the tannin, the only other inactive component of great importance is the saponin fraction."

To give an insight into the physical and chemical properties of the substance with which he dealt, we quote further from his article—"The marc left after extracting the leaf with chloroform was treated with many different menstrua, chiefly various dilutions of alcohol, in an attempt to determine which extractive was best suited to remove the residual activity." "It was found that the amount of active matter was identical in extracts made with any percentage of alcohol from water to 75%, but that if stronger alcohol were used, extraction was apt to be incomplete." "The total amount of solids removed was fairly constant with all dilutions until concentrations above 75% were reached. Thus from the same lot of leaf, 5% alcohol removed 425 grams of solids per kilo; 75% removed 430 grams; while 95% removed only 125 grams. The difference in the amount of solids is mainly due to the tannins which are not soluble in strong alcohol, and can be precipitated from extracts containing them, by merely increasing the percentage of alcohol."

It was this last statement, together with the one on the amount of tannin, which aroused special interest. The matter was discussed with Dr. Githens, who said that his estimation was based on the amount of a precipitate thrown out of water solution upon the addition of several volumes of 95% alcohol, and that this precipitate, when redissolved in water, showed a green color with ferric chloride. Thus attributing this reaction to tannin, he used the term merely to tell of this material in contrast with the active ingredients with which alone he was concerned,

and without intention to definitely designate the precipitated matter as tannin. Therefore, to the term as used by him, he attached no other significance than that of the iron-greening behavior of this constituent.

Dr. Githens welcomed further consideration of the subject and expressed the hope that effort would be made to isolate and purify the iron-greening factor. Accordingly this was undertaken.

In contemplating the likely occurrence of tannin in digitalis, certain facts of practical value are particularly worthy of review. For instance, while every plant material which contains tannin has not been listed as a source of this agent, still there are few drugs as well known as digitalis that have not been thus indexed if they show even a few per cent. of astringent principle, so little as 5% attracting attention. But, though the reaction of infusion of digitalis with iron salts has long been known, the drug is not found in such lists of plant substances; which is not at all strange because neither the taste of the leaf when chewed nor that of its infusion suggests astringency. The United States Pharmacopœia, without further comment, describes the taste of digitalis as strongly bitter. Nor is there any qualification of this property by other authorities, except that some refer to the bitterness as nauseating. However there is, at times, a sensation of benumbedness experienced from tasting strong decoctions, which might be fancied as astringency almost completely masked by bitterness.

Also, it may well be recalled that solubility in alcohol has been noted as a constant character of the tannins, even of those insoluble in water; for which reason, the tannins are not regarded as precipitable from water solutions by addition of alcohol in any amount.

But since some very unexpected results have been realized while working with tannins, surprises may well be expected; and, during the last few years, experience has shown that not even taste can be depended upon as a guide, for the tannins of red rose petals and wild cherry bark are examples of these principles which are bitter as well as astringent. Experience has clearly revealed the need to isolate and purify every such plant principle as the way to an understanding of its behavior in galenicals.

A number of preliminary experiments were made, during which the presence of an iron-greening constituent was abundantly demonstrated.

Extractions of the leaf with both hot and cold water and with different dilutions of alcohol substantiated the statement of Githens with reference to the extent of solvent action of varying percentages of alcohol, but did not show the iron-greening factor to be insoluble in official alcohol. The precipitating effect of strong alcohol on aqueous and weak alcoholic extractions was also confirmed, but it was found upon washing the precipitate with alcohol, dissolving in water and reprecipitating with alcohol, that the precipitated matter gradually lost its iron-greening effect. Whitish or rusty-looking gelatinous precipitates are produced by acetone just as by alcohol.

Estimations of the "tanning value" of digitalis were made by use of the "hide-powder" method. The hot water infusion of one lot of drug showed 23.33 per cent. and that of another lot 26.66 per cent. A cold water infusion of the latter lot gave figures to indicate 30 per cent. The solids removed by hide represented from about two-thirds to three-quarters of the total soluble solids.

The effect of the rasped raw hide on these infusions was remarkable. Originally, dark colored, with heavy odor, strongly acid to litmus, very bitter and giving much dark green color with ferric chloride, contact with hide powder for a few hours, followed by straining and filtration, showed that all but traces of color, odor, acidity, bitterness and iron-greening effect had been absorbed. When the liquid was again treated with hide powder and again expressed from it and filtered, it was found to have lost all color reaction to ferric chloride. This behavior distinctly proves the absence of gallic acid; conditions to detect which were here proved as proper by control of test.

So far as removal of acidity by hide is concerned, comparisons by actual titration indicated that as much as 95% of the original acidity had been withdrawn from the liquid. In some instances the hide-treated solutions gave precipitates with lead acetate, with others this did not occur or was but slight. Under the influence of hide, the behaviors of hot and cold water infusions were little, if any, different. By fractional application of the hide, it was found that the absorption of the several properties is gradual. Infusions of digitalis may or may not precipitate gelatin solution; the addition of acetic acid, which has been found to facilitate such precipitation with some tannin materials, was not uniformly dependable with these infusions.

The large amount of solids absorbed by the hide led to the adoption of half-kilo quantities of the drug for extraction with the several solvents which were decided upon for trial in methods that have been successfully employed for isolating and purifying tannins.

One such portion was exhausted with cold water, a second with boiling water, a third with chloroform and then with acetone, a fourth with acetone only, and a fifth with acetic ether. Chloroform removed about 3.88 per cent. of dark green, fatty or oily extractive. Acetone, applied thereafter, took out about the same amount of solids. While this extractive contained chlorophyll, it was of a very different texture from that of the chloroform extractive. The acetone extractive yielded little to ether, but was completely soluble in acetic ether, and largely soluble in water.

Every one of the foregoing named solvents removed from digitalis the cause of the iron-greening reaction. But upon the following out of the usual plan of treating the extracted matter with water, shaking out with acetic ether, and purifying the substance so removed by trituration with ether and chloroform, only a trifling amount of the half-kilo of material started with was found to have followed through. During these experiments it was learned that neither acetic ether, ether nor chloroform completely washed out the iron-greening factor from water solution. All of these solvents removed some of the substance, acetic ether extracting it more readily than ether, chloroform less readily. Consequently all phases of the drug material gave the characteristic reaction with ferric chloride. In the substance removed there was much of a white crystalline acid principle that tenaciously accompanied the slight amount of matter which followed through the above outlined process. The final product from half-kilo portions of the drug had a light straw color, but was little more than a few blotches on the glass of the flask in which it was recovered from solution in acetic ether or alcohol.

The relatively larger amount of solids absorbed by hide made this result all the more peculiar.

The several half-kilo portions having failed to give an appreciable yield, 4 kilos of ground *digitalis* were percolated with acetone until exhausted. The extractive from this lot amounted to about 6.66%. It apparently contained the greater part, if not all, of what chloroform removes from the drug.

This treatment with acetone took out much of the iron-greening factor, but not all of it. It had been found by preliminary experiments that acetone, like alcohol, has a precipitating effect upon certain of the constituents; therefore, as with alcohol, it is reasonable to infer that acetone, too, fails to completely extract that fraction of the iron-greening factor which is thereby enveloped and retained by the marc. This indeed is the explanation of the smaller amount of extractive by strong alcohol, but which extractive contains abundance of the iron-greening factor.

After the marc from the acetone extraction had been deprived of adhering solvent, a portion of it was treated with cold water. Six successively applied lots, each followed by expression, practically accomplished exhaustion of this material in so far as color, taste, acidity and iron-greening reaction served as guides. The several watery solutions showed these features in gradual diminution from the first with much color, odor, acidity, bitterness and iron reaction down to an almost complete absence of these properties. These liquids were mixed and evaporated to about twice the original drug volume, then mixed with six volumes of acetone, which produced a decided turbidity due to a precipitate which gradually settled into a reddish amber, viscid, gum-like sediment. The liquid retained its original dark amber color. Next, the marc was boiled with water, which treatment also removed soluble solids, but much less than those by cold water. The hot water extractions were concentrated and mixed with acetone as was the cold water treatment. It, too, yielded a viscid sediment similar to that from the cold water analogue, but less.

An examination of these viscid precipitates and of the liquids in which they were produced, showed that the iron-greening substance tended to remain in solution rather than to precipitate with acetone. It is therefore in the precipitate by inclusion and not because of insolubility.

These gum-like precipitates were insoluble in acetic ether and alcohol, but readily soluble in water, which solution yielded nothing of account to acetic ether. Neither the water solutions of these substances nor the original water extractions from which they were obtained, showed any change in color with iodine test solution.

Returning to a consideration of the acetone extractive, this substance was treated with chloroform, which took up the greater part of the chlorophyll and left a reddish brown, plastic mass. This was freed of chloroform by warming and then treated with water in which it was almost entirely soluble. The solution had a dark amber color, was very bitter, strongly acid to litmus and reacted abundantly green with ferric chloride. The water solution, clarified by aid of paper pulp, was shaken with acetic ether which removed considerable amount of reddish amber, semi-solid matter, as shown upon recovery of solvent. When this residue became cool, crystalline substance was plainly discernible in the mass. It was noted that the later portions of the acetic ether were removing proportionately more of the crystalline matter than of other substances. By recrystallizing the matter taken

out by further application of acetic ether, using this same solvent for the process, pure white crystals of an acid principle were finally obtained. When the crystals of this acid are untinged with yellow, they do not give a green color with iron solution, but do precipitate lead acetate of a canary-yellow. When free from yellow color, these crystals are sour but not bitter.

The material taken out of the water solution by acetic ether, and left as a mixture of crystalline and non-crystalline matter, was treated with several successive lots of ether, which gradually took away some of the crystalline matter as also a yellow substance which seemed to be amorphous. But the removal of these substances appeared to progress only to a certain point and there stop, after which re-resolution in water and again shaking out with acetic ether had the effect of again inducing ether to remove additional yellow color, but little of the crystalline matter. Chloroform was serviceable in the first few repetitions of this water treatment, by removing green and yellow coloring matter; thereafter it was without effect.

In the early stages of this treatment of the acetone extractive, some considerable amount of a somewhat porous residue, in appearance very much resembling a tannin, was left upon evaporating, *in vacuo*, the acetic ether with which this substance had been taken from water solution. But the next application of water showed the greater part of this residue to be insoluble.

To guard against loss here of tannin insoluble in water, like that found in matè, the insoluble portion was at once taken into acetic ether solution, reobtained by distilling off the solvent, and subjected to repeated treatment with ether and chloroform. These solvents continued to remove small amounts of soluble matter, until the mass was again mostly soluble in water. To further protect against loss of tannin of this peculiar nature, all water-insoluble residues were checked up by similar treatment. These formations were mainly of fatty, waxy, chlorophyl and resinous substances. The care that was taken throughout the process warrants the belief that no substance of the usual tannin nature was anywhere lost or set aside. The chloroform-soluble part of the acetone extractive was worked through the process for tannin; it showed nothing of such nature beyond small amounts of the iron-greening factor.

Finally, by the process outlined, an almost colorless product, that appeared to be little if any changed by further use of solvents, was had. The tendency to form a porous residue upon evaporation of solvent had by this time much diminished; still there was such tendency left in the product, though when the liquid was approaching dryness and there was for a moment a froth-like formation at the edge of the liquid which spread into the area of the residue as evaporation went on, there was a change in appearance as the last few drops of solvent were vaporized, the mass coalescing into a pellucid-punctate, straw-colored film upon the flask.

Although this educt gives the characteristic reaction with ferric chloride, and has withstood all attempts to further purify it, it is not regarded as a single substance. It contains crystalline substance, which at times has been unmistakably visible.

A second lot of 4 kilos of digitalis was exhausted with acetone, and the solids thus removed treated at once with water, without the intervention of chloroform. The experience with this lot was but little different from that had from the previous method. The interferences and outcome were about the same.

With the thought of excluding some of the substances which proved to be insoluble in water, a third lot of 4 kilos of the drug was exhausted with hot water; the decoctions were mixed, concentrated, cooled, strained and shaken with acetic ether. Separated and evaporated, this solvent left a reddish amber semi-fluid residue, that, upon treatment with ether, chloroform, acetic ether and water as customarily used for the purpose, gave an educt identical in appearance and other general properties with those from the acetone extractions.

Since similar results were had by operating on kilo lots of the drug with cold water, it would seem that boiling the solution of the soluble solids does not influence the final product.

Dialysis was turned to as a possible means of isolation and purification. Some very concentrated decoction was placed in an animal parchment bag and this suspended in sufficient water to submerge the entire bulk of contents. It was soon demonstrated that the yellow color, the bitterness, the acidity and the iron-greening substance were passing through the membrane. The water was frequently changed. Eventually the osmosis was complete, all bitterness and all iron-greening reaction having passed through, and only the slightest herbaceous taste, without suggestion of astringency, was left in the liquid remaining within the septum. This experiment was made several times, with the same result. This was found to be the case with the isolated material as well as with the decoction. Being flatly contradictory to the effect of hide-powder, this experience with the dialysis of solutions of the iron-greening factor becomes intensely interesting.

The educts from the several methods of isolation and purification gave the characteristic iron-greening reaction. They were all sparingly or slowly soluble in cold water, more readily in hot; the solutions displayed amber colors, possessed very bitter tastes, had strong acid reactions to litmus. Their solutions were precipitated by lead acetate, and by gelatin. Hide-powder removed all of these attributes, except a slight taste and some greatly reduced precipitating effect with lead acetate. They were soluble in alcohol and acetone; insoluble in ether and chloroform. They were dialyzable from pure water solutions just as from decoctions. The substances dissolved in alkalis (ammonium, potassium and calcium hydrates) with production of deep yellow colors. These solutions were rendered colorless by acidification; there was also precipitation during this change.

If this educt is regarded as a tannin it is a most unusual one. The chief point in evidence that this iron-greening principle might be a tannin is the fact that hide-powder completely absorbs it and that it precipitates gelatin—two properties, at present, authenticated to all tannins, but which, in the light of the equally patent fact that this iron-greening factor passes through animal membrane, need to be better understood before the presence of tannin in digitalis or its absence therefrom can be conclusively decided.

To summarize results, it may be said that:

The matter precipitated by alcohol is not responsible for the iron-greening behavior; it merely envelops some of the cause of this. The iron-greening factor is soluble in 95% alcohol.

Digitalis contains colorless, bitterless, crystalline acid substance which gives no change in color with ferric chloride.

Digitalis does not contain gallic acid, gallotannic acid or tannins like those found in oak bark, pecan nut, red rose, matè or wild cherry.

While the iron-greening factor is completely absorbed by hide-powder, it is equally capable of passing through animal membrane; which contradictory behaviors at once raise that continually recurring enigmatic question—What is a tannin?

To avoid adding to the existing confusion, it is suggested that, until more is known of this anomalous constituent of digitalis, the same be referred to as the iron-greening factor.

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### THE QUANTITATIVE DETERMINATION OF CAMPHOR IN PHARMACEUTICAL PRÉPARATIONS.

BY H. VIETH AND E. A. BILHUBER.

Though camphor is used to a considerable extent in the field of medicine there is no dependable method recorded for its quantitative determination in the small amounts in which it occurs in pharmaceutical preparations.

The usual method employed is based upon the degree of rotation of a camphor solution as determined by means of the polariscope. This method, given in the Pharmacopœia, can only be used for the natural dextro-rotatory camphor. Furthermore this method for the determination of camphor is not often practical for the analysis of pharmaceuticals, principally because relatively large amounts of camphor are required while the usual preparations contain but very small quantities. All the added ingredients and inerts that far outweigh the camphor present in most such compounded products detract from the accuracy of the polarization or from the simplicity of its application. The usual camphor preparations, tablets or pills, contain but from 0.05 to 0.1 Gm. camphor and at least 1.0 gram of camphor; that is, 10 cc. of a 10% solution absolutely free from foreign matter, is necessary for an exact polarization determination. More dilute solutions than given above permit too great possibilities of error in the readings. Therefore, this method may well be used for the determination of camphor in the official Spiritus Camphoræ but is, in general, impractical for other preparations.

Other methods have been proposed, but have given unsatisfactory results. Thus Fuller gives a method in which he converts the camphor into its oxime with hydroxylamine and then titrates. Gildermeister proposed to determine camphor by oxidation with permanganate. Not only is this method not quantitative, but it requires larger amounts of camphor.

The natural procedure for the determination of camphor in small quantities in pharmaceutical preparations would be to isolate the pure camphor and weigh it as such. In the development of such a method it was found that the rapid volatilization of camphor had to be controlled.

Preliminary trials were made on the rate of evaporation of camphor and it was found that the pure powder loses about 10% in weight daily when exposed to the usual room temperature. It must therefore be weighed in a closed vessel. A solution of 0.5 Gm. camphor in 30 cc. chloroform contained only 0.26 Gm. after it had stood 48 hours in an open vessel at room temperature. Only the slightest trace of