Texas, and then south to Mexico. There are some trees further north, but these are said to possess a lower balsamic content. The trees of the American species measure when well matured from five to twelve feet at the base, and often attain a height of 150 feet. The wood is exceedingly hard, and straight, close-grained, but not very strong. The roots spread widely and rapidly, and deeply under the ground. The sapwood has upwards of sixty layers of annual growth.

Among the common names for American styrax are sweet gum, gum wax, liquidambar, and copalm balm. Levant styrax has lately been quoted at $8.00 a pound, and very little on the market; American styrax is not quoted regularly; it should even now be produced at a figure less than half of this. In contrast to the Levant species the American variety seems to be exempt from insect injury. The outlook for larger use of American styrax seems encouraging.

STUDIES ON THE PHARMACOLOGY OF DIGITALIS BODIES.

I. THE ACTIVITY OF THE DIGITALIS GLUCOSIDES.

BY THOMAS S. GITHENS.*

In 1910 I began studies in this laboratory with a view to making a preparation of digitalis which would avoid as far as possible the irritating and nauseating properties of the crude drug, while retaining its entire physiologic and therapeutic powers. Experiments were made with the two usual methods of preparing digitalis glucosides; namely, the precipitation of inert matters by lead acetate; leaving the active glucosides in solution; and the precipitation of the glucosides by tannic acid followed by their further purification. It was, however, found that both of these methods destroyed a large proportion of the active constituents and that the glucosides which remained were altered in some obscure manner, so that they no longer represented the drug properly. An attempt was therefore made to separate and purify the active constituents without the use of powerful chemical agents.

In order to understand the problem presented it is advisable to describe briefly the chief constituents of digitalis, active and inert. Digitalis contains at least four active glucosides, of which only two are of importance; namely, digitoxin and digitalein. Gitalin, described by Kraft[1] as the chief active principle, was established by later work of Kiliani[2] to be a mixture of glucosides, altered by chemical manipulation. Digitoxin and digitalein resemble each other closely in physiologic and therapeutic action, but differ widely in solubility, absorbability and in power to resist destructive agencies. Digitoxin is freely soluble in alcohol, even more soluble in chloroform, but insoluble in pure water. It is, however, soluble in a solution of the saponin which is present in digitalis leaf and it is therefore extracted from the leaf by water. Digitalein is insoluble in chloroform and ether, freely soluble in water, and sparingly soluble in alcohol. It is well extracted from the drug by alcohol, only if the drug be powdered. Its solubility in alcohol is

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[1] F. Kraft: *Arch. der Pharm.* 250, 118, 1912.

[2] H. Kiliani: *Ibid.*, 252, 13, 1914.

increased by the presence of dilute alkalies, which, however, do not seem to alter it, and it is dissolved by mixtures of alcohol and ether in the presence of alkali.

Digitoxin and digitalein differ, furthermore, in their resistance to destructive agencies. The former is not materially affected by treatment with lead acetate and can be precipitated by tannin and recovered from the combination, without loss of its physiologic activities. It is also relatively resistant to heat. Digitalein, on the other hand, is very sensitive to strong chemical agents. If a preparation containing it is treated with lead acetate, it will be found that it has lost from one- to two-thirds of its activity. It cannot be recovered in an uninjured form from the combination which it forms with tannin. In addition it is readily injured by heat, temperatures above 45° to 50° C. reducing its activity markedly.

An average, good specimen of leaf contains about 0.25 percent of digitoxin which lends the leaf from one-third to two-fifths of its activity. The amount of digitalein is not readily determined by chemical tests but, judging from partially purified specimens, there is about 0.5 percent. As it is slightly less active than digitoxin it accounts for three-fifths to two-thirds of the activity of the average leaf. The proportions of the two glucosides vary, in extreme cases, within much wider limits. The digitoxin may account for more than half the activity of the drug or may be almost entirely wanting.

It may not be superfluous to point out that many statements in regard to the chemistry of the leaf in current textbooks are at fault owing to the failure to realize that the chemistry of the leaf differs widely from that of the seed. The chief active constituent of the seed is a glucoside, digitalin (*digitalinum verum*); small amounts of digitalein are also found, but there is no digitoxin. The chief other component is a saponin, digitonin, which is not present in the leaf. The saponins of the leaf are often incorrectly spoken of by this name.

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 percent 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. Beside the tannin, the only inactive component of great importance is the saponin fraction. This consists apparently of a mixture of several saponins which are not readily separated from each other or from the active glucosides. The saponins are of great pharmaceutic importance, as digitoxin is soluble in an aqueous solution of saponin and through its presence the digitoxin is present in aqueous preparations of the leaf, such as the infusion. The saponins are not only extremely irritating topically, but are hemolytic.[1]

CHEMICAL STUDIES.

The differences in the solubility of the two active glucosides, and the impossibility of treating the water-soluble ones with strong chemical agents, made

[1] In view of several recent statements that the saponins of digitalis leaf are not hemolytic, I would add that saponins separated as described below caused complete hemolysis of 1 Cc. of rabbit blood in amounts containing only 2 mg. of solids and corresponding to 8 mg. of leaf. The tubes were kept one hour at room temperature in hot weather. According to McGill (*Jour. Am. Chem. Soc.*, 42, 1900, 1920), this action depends on impurities.

it evident that better results were likely to be obtained in their purification if they were first separated. Attempts were made to remove the digitoxin from the digitalein by first extracting the drug with dilute alcohol, and after driving off the alcohol, shaking out with chloroform. This method, modified in several ways, did not lead to complete extraction of the digitoxin and an attempt was therefore made to extract the drug first with chloroform and then to obtain the digitalein by extracting the marc left after this preliminary extraction. This method proved to be so thoroughly satisfactory that it was used in all later studies.

THE CHLOROFORM EXTRACT.

If digitalis leaf, powdered finely, is extracted with chloroform, a dark green fluorescent liquid is obtained, which, on evaporating off the chloroform at moderate temperature, leaves a dark green, very sticky and greasy mass, with a characteristic digitalis odor. This extract is partly soluble in benzine and a larger portion is soluble in ether. It is almost completely soluble in hot alcohol but not readily taken up by cold. It represents about 3.5 percent of the total weight of the dried leaf and contains, in addition to the digitoxin, chlorophyll, which gives it its color; fats; a fixed and a volatile oil; free fatty acids, and coloring matters. As most of these substances are not soluble in dilute alcohol while the active principle is, the material may be purified by dissolving in hot alcohol and treating with an equal volume of water. There results, on filtration, a somewhat turbid yellow liquid which cannot be filtered clear through filter paper, and cannot be treated with kieselguhr as this adsorbs digitoxin (in contrast to digitalein). A perfectly clear and satisfactory solution may, however, be obtained if 1 percent of alum is added to the water before adding it to the alcohol solution. Alum is very slightly soluble in 50 percent alcohol, and almost all the alum is thrown down with the solution and filtered off. As it was felt that this process gave an undesirably high percentage of alcohol, it was later modified by dissolving the chloroform extract in a smaller amount of hot alcohol and adding four volumes of water. The solution obtained on filtering this mixture is clear, light yellow, and smells of digitalis. An average lot contains about 1.4 grammes of solids for every hundred grammes of leaf. The activity of a good specimen is such that less than 2 mg. are required to kill a guinea pig weighing 250 grammes, while 0.02 mg. per gramme will stop the heart of a frog (*Rana pipiens*) within an hour.

THE WATER-SOLUBLE GLUCOSIDES.

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 percent, 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 percent were reached. Thus, from the same lot of leaf, 5 percent alcohol removed 425 grammes of solid per kilo; 75 percent removed 430 grammes; while 95 percent removed only 125 grammes.

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 contain-

ing them, by merely increasing the percentage of alcohol. The solubility of tannins in alcohol is decreased by alkalinity, while the solubility of the active principles is increased. It is thus possible to bring about extraction under more favorable conditions, and to obtain an active extract almost free from tannin, by extracting with 95 percent alcohol rendered strongly alkaline by ammonia vapor. An extract prepared in this way still contains the saponins, which are soluble in alcohol even in the presence of alkalies.

The saponins may be separated from an alcoholic solution containing both them and digitalein, by adding ether, which throws them down, but leaves the glucosides in solution. It seemed possible that both the tannins and saponins might be removed from the glucosides by treating a dilute alcoholic solution with ether. This forms layers, an alcohol-water layer below and an alcohol-ether layer above. There is no tendency to form emulsions. Trial proved that this method was feasible. The best proportions were found to be 45 parts of water, 55 parts of alcohol and 120 parts of ether. With this mixture, the lower layer, which measures less than 45 parts, contains almost all of the tannins and saponins, while the upper layer contains all the active matters. This separation is sharp, only when the reaction is alkaline, which is best provided for by adding a sufficient amount of strong ammonia water.[1]

In view of the solubility of the active principles in a mixture of alcohol and ether, attempts were made to extract the chloroform marc with such a mixture. Experiments showed that a satisfactory extraction could be accomplished with a mixture consisting of alcohol 10 parts, ether 40 parts and strong ammonia water 1 part. After complete extraction, which requires less than 10 volumes of the mixture, the extract is treated with one-tenth volume of water, which forms a small layer. This takes out almost all of the small amounts of saponin and tannin which have been extracted, as well as almost all the excess ammonia. The upper layer from this extraction is evaporated almost to dryness *in vacuo* and the residue treated with water. A turbid, pale yellow solution results which cannot be cleared by filtration through paper but can be filtered through kieselguhr or through an earthen filter candle without loss of activity. The solution obtained in this way contains all the activity remaining in the chloroform extracted drug but contains very little extractive matter. A properly made specimen should kill a 250 Gm. guinea pig in a dose which contains only 1 to 2 mg. of solids.

This solution of the watery principles has shown very little loss of activity in six months, but on account of the absence of alcohol in the finished preparation, a preservative must be added to prevent growth of fungi, to which it is very liable. Addition of 0.5 percent sodium benzoate has been found more suitable for this purpose than other preservatives which were tried.

PHARMACODYNAMIC STUDIES.

Both the preparations just described, *i. e.*, the digitoxin and digitalein, have been used in a large series of experiments on animals with a view of determining whether each of them represented the full activity of digitalis. It was found

[1] Githens, *Jour. Pharm. & Exp. Ther.*, 15, 245, 1920.

that their action, as judged by qualitative studies, is identical. Both kill guinea pigs in small doses and the symptoms preceding death, such as nausea and convulsions, are the same. They raise the blood pressure of the rabbit to about the same extent and slow the heart equally. Both show an uncertain diuretic action; the amount of diuresis and the number of animals exhibiting it do not differ much. Both substances cause strong contraction of the isolated uterus and the amounts required to produce a given degree of contraction are not very different. It may therefore be stated that their actions are apparently qualitatively identical.[1]

While the above work was under way an important article on the relative actions of the chloroform- and water-soluble glucosides of digitalis appeared from the laboratory of Professor Robert A. Hatcher.[2] Dr. Hatcher has shown that the most important difference between these glucosides lay in their absorbability in the digestive tract and their liability to destruction in the stomach and bowel. He found that the chloroform-soluble glucoside, that is to say, the digitoxin, was readily absorbed from the gastroenteric tract and developed its full activity when administered by mouth; the water-soluble glucoside, on the other hand, that is, the digitalein, was poorly absorbed from, or largely destroyed in the digestive tract, so that when it was administered by mouth it showed very much less activity than when injected subcutaneously or intravenously.

It is evident from these facts that if a mixture of the two principles is administered by injection the full effect of each will be developed and the total effect will be the sum of the separate activities. If, on the other hand, such a mixture be given by mouth, the digitoxin only will develop its full effect, and the activity of the preparation will be less than would have been anticipated. If the preparation or the crude drug containing such a mixture happened to be rich in digitoxin, the activity by mouth would approach that by injection, whereas if it were relatively poor in digitoxin, the effect would be much less than might have been anticipated from the results of the drug given hypodermically. The importance of these considerations is dependent on the fact that all preparations of digitalis on the market are standardized by subcutaneous injection (in frogs or guinea pigs) or by intravenous injection (in cats). It is obvious that if any preparation is unduly poor in digitoxin and rich in digitalein, the physiologic assay will show it to be much stronger than it will later appear in the clinic when administered by mouth.

From the facts shown by Dr. Hatcher it is plain that a preparation containing only the digitoxin is more suitable for administration by mouth than a preparation containing the total glucosides of the leaf. On the other hand, it is well known that digitoxin is much more irritating topically than digitalein and for this reason a preparation containing only the latter is better adapted for hypodermic administration. Preparations made by the methods described would seem, therefore, to be better adapted to clinical use than the usual preparations containing both glucosides.

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[1] Githens, *Jour. Pharm. & Exp. Ther.*, 15, 239, 1920.

[2] R. A. Hatcher, and C. Eggleston: *Ibid.*, 12, 405, 1919.