SCIENTIFIC SECTION

A PREPARATION OF DIGITALIS SUITABLE FOR INJECTION OR ORAL ADMINISTRATION.*

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The Council on Pharmacy and Chemistry of the American Medical Association, at the annual meeting, April 6 and 7, 1928, discussed the desirability of admitting a preparation of digitalis suitable for hypodermic injection to the U. S. Pharmacopœia, and the Council offered assistance to the Pharmacopœial Committee of Revision in studying the activity of a preparation of digitalis for hypodermic use. Prof. E. Fullerton Cook, Chairman of the Committee of Revision, suggested that the Council undertake an investigation of Liquor Digitalis ad Injectionem, or Digisol for Injection, which is official in the Netherlands Pharmacopœia V.

No definite problem was submitted to us by the Council on Pharmacy and Chemistry or by the Chairman of the Committee of Revision, hence, we selected from the many questions presenting themselves several which gave promise of a ready solution. We have therefore investigated the following: (1) methods of preparation; (2) emetic activity; (3) absorbability; (4) persistence of action.

The following discussion is based on the results of experiments in which we used 15 specimens of digitalis, varying in activity from the weakest obtainable to the most active. A total of some 226 cats was used in the investigation. No complete protocol is given for the reason that single experiments often involved a variety of determinations, such as the period of extraction required, method of purification of the residue, absorbability, emetic activity and other factors. We are therefore able to give only the results of these experiments in condensed form, with a discussion of their significance.

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I. METHODS OF PREPARATION.

A. The Method of the Netherlands Pharmacopœia V.—Professor Cook kindly supplied a nearly literal translation by Dr. D. Twiss of the directions for making digisol which is practically like that given by Lewis G. Freeman (1). The following is the translation, slightly abridged:

Add eight parts of water to one part of digitalis No. 30 powder, shake repeatedly during 48 hours at a temperature not above 15°, strain through cloth, allow to settle, decant the clear liquid, filter the rest and add the filtrate to the decanted portion. Shake the watery extract with an equal volume of chloroform in a nearly filled flask, repeatedly and vigorously at intervals during 48 hours, avoiding emulsification so far as possible.

Collect the chloroformic layer; concentrate a certain volume of the watery portion to a thick extract on a water-bath; mix this extract with enough dried sodium sulphate so that pulverization will yield a homogeneous dry powder; shake this with a volume of the collected chloroformic extract equal to that of the aqueous liquid that was concentrated, repeatedly and vigorously at intervals during 24 hours;[1] filter; determine the volume of the chloroform, and distil; treat the residue repeatedly with a volume of water (to be added in portions) equal to that of the chloroform distilled; dissolve 0.8 per cent sterilized sodium chloride in the liquid, filter and sterilize in convenient containers by heating on three successive days for one hour, at 70–80°.

The clear, almost colorless or slightly green-yellow liquid, having an odor of digitalis, has a very bitter taste and foams strongly on shaking. Tested as described for digitalis leaves, a lethal dose should be 2 cc. per Kg. of cat. When 1 cc. digitalis liquid is evaporated and the residue dissolved in 1 cc. concentrated acetic acid, the solution should, after the addition of a trace of ferric chloride, when poured on concentrated sulphuric acid, show a red-violet coloration of the border layer above which a blue-green coloration is observed.

An essentially similar preparation for internal use, containing 12 per cent of alcohol, is also official.

The products of the several stages of the process are indicated for convenience of discussion, as follows: "aqueous filtrate," that obtained after macerating the digitalis with 8 parts of water w/v; "first chloroformic extract," the chloroformic portion separated after shaking the aqueous filtrate with an equal volume of chloroform; "first aqueous residue," the aqueous portion remaining after the separation of the first chloroformic extract; "first chloroformic residue," that obtained after distilling the first chloroformic extract; "second chloroformic extract," the chloroformic portion obtained after evaporating the first aqueous residue, adding dried sodium sulphate, drying, powdering and extracting the powder with the first chloroformic extract; "second chloroformic residue," that obtained after distilling the second chloroformic extract; "fresh second chloroformic extract," the chloroformic portion obtained by evaporating the first aqueous residue, adding dried sodium sulphate and treating the powder with fresh chloroform, instead of using the first chloroformic extract. In some cases the second chloroformic extracts and residues were prepared without evaporating the aqueous residue.

[1] These directions seem fairly concise. Nevertheless, we received another translation directing that the first chloroformic extract be discarded, and at least one prominent manufacturer informed us that he had interpreted his translation in that way. In order to settle the matter authoritatively, we wrote to Professor W. Storm van Leeuwen, of Leiden, who replied that Professor Bijlsma—the successor of Magnus—stated that a portion of the first chloroformic extract is used later for the extraction of the dried portion.

a. Period of Aqueous Extraction.—The formula of the Netherlands Pharmacopœia is closely similar to that recommended by Sluyters (2) for the extraction of both gitalin and digitalein,[1] except that Sluyters extracted digitalis with water during 24 hours, and he does not specify the period of agitation with chloroform.

In order to determine whether it is necessary to shake the powdered digitalis with water during 48 hours, as the Netherlands Pharmacopœia directs, the following experiment was performed. One hundred fifty grams of digitalis was divided into three equal portions and extracted in the following manner: (1) during 48 hours; (2) during 24 hours; (3) during 2 hours. The purified residues, each from 100 cc. of the first chloroformic extract, were: (1) 35.5 mg., (2) 34.0 mg. and (3) 21.0 mg. The tests showed that the total activity of the purified chloroformic residue obtained after extraction during 24 hours was almost exactly the same as that after 48 hours, hence nothing is gained by the longer period of extraction. The slight yield after aqueous extraction during two hours indicates that this period is insufficient. It is in marked contrast to the rapid extraction with hot water.

b. Period of Chloroformic Extraction.—Several experiments were performed in order to determine the necessary period of extraction of the first aqueous filtrate with chloroform. One hundred twenty-five grams digitalis, of which 100 mg. equalled 1 cat unit,[2] was extracted in the manner directed, during 48 hours, after which 620 cc. of filtrate was obtained. This was divided into two portions. The first portion of 120 cc. of aqueous filtrate was rotated gently with 120 cc. of chloroform in a flask having a capacity of about 500 cc., during two hours; 100 cc. of the chloroformic distillate yielded 41.6 mg. of residue. The second portion of 500 cc. of the aqueous filtrate was rotated gently at intervals during 48 hours, with 500 cc. of chloroform in a flask having a capacity of about 2000 cc. The residue left after the distillation of 100 cc. of chloroformic extract weighed 40.5 mg., or almost exactly the same as that after shaking during two hours.

In another experiment, equal volumes of aqueous filtrate, prepared in the manner described, were shaken with chloroform, as follows: (1) with gentle rotation at intervals during 48 hours; (2) vigorously at intervals during 48 hours; (3) vigorously to form an emulsion several times during 48 hours; (4) vigorously at intervals during 1 hour with complete emulsification. Equal volumes of these four chloroformic extracts yielded practically similar amounts of residue.

It is obvious that the rate of removal of a principle from one liquid by shaking it with another with which it is immiscible depends on several factors including: the solubility of the principle in the first liquid (water); the solubility in the second liquid (chloroform); the concentration of the solution; the degree to which the two liquids are brought into contact, that is, the extent and duration of surface contact in proportion to the total volume. The following illustrates certain of these facts.

Strychnine base is soluble in about 6420 parts of water and in about 7.5 parts of chloroform. When a 1 per cent solution of strychnine sulphate is made alkaline

[1] The composition of digitalein is so variable that the name has no fixed meaning.

[2] The cat unit, described by Hatcher and Brody (3) is that amount of a digitalis body which is required for each Kg. of weight to cause death when it is injected slowly into the vein. We refer to it as the average fatal dose.

by the addition of sodium bicarbonate and is shaken vigorously during one minute with an equal volume of chloroform in a separatory funnel of a capacity twice that of the volume of the combined liquids, all but a trace of the strychnine is removed by the chloroform. On the other hand, morphine base is soluble in about 3340 parts of water and in about 10,000 parts of absolute chloroform (not the official), hence, it requires a very large excess of absolute chloroform to remove all the morphine base from an aqueous solution.[1]

The Netherlands Pharmacopœia directs the shaking of the aqueous filtrate with chloroform in a nearly filled bottle, in order to avoid emulsification, so far as possible. We have found this warning entirely superfluous. It requires little skill to rotate a half-filled flask without forming much emulsion, and usually even after vigorous shaking, the greater part of the chloroform separates on standing some hours, and one can easily separate nearly all the chloroform from the emulsion, which consists almost wholly of chloroform. The emulsion is split readily by shaking it with five volumes of fresh chloroform, or, if one prefers, with the chloroformic extract previously separated. In an experiment in which three portions of 125 cc. each of filtrate were shaken vigorously with chloroform, 124 cc. of the chloroformic extract was recovered in each of two portions, and 110 cc. in the third.

Vigorous shaking in a half-filled vessel breaks up the liquids into small globules, increases the surface areas in contact, and facilitates extraction correspondingly. If a vessel is completely filled with equal volumes of chloroform and the aqueous filtrate, the most vigorous shaking does not break up the two liquids into small globules and the surface areas in contact are increased comparatively little.

The deeper the layers of water and chloroform, the less are the areas of the surfaces, hence, the more vigorous is the shaking required, or the longer the contact for complete extraction. If the chloroformic and watery layers are rotated in a nearly flat vessel of such diameter that the depth of the two liquids does not exceed one or two centimeters, extraction requires only gentle rotation during an hour or two. The more nearly the shape of the vessel approaches to a cylindrical form and the deeper the layers of the liquid, the longer the time required, or the more vigorous the shaking, for extraction, and the figures which we give apply only to conditions approaching those which we have used.

The surface area of one liter of chloroform in a flask having a capacity of 5 liters measured 314 square centimeters, and the depth of the aqueous layer was approximately $3^1/_2$ centimeters. When one rotates a liter of the aqueous filtrate with a liter of chloroform in this flask, the extraction is completed within two hours, but if one were to place the chloroform and the aqueous filtrate in an ordinary bottle of about two liters' capacity, leaving an air space of only a few cubic centimeters, extraction would be very much slower and much more vigorous shaking would be required.

c. Evaporation of Aqueous Residue and Extraction of Powder.—There are several factors in the preparation of the second chloroformic extract that require consideration. Care is taken to maintain the temperature at 15° or lower until the first extraction with chloroform is completed, after which the aqueous residue

[1] Chloroform containing about 4 per cent of alcohol by volume is a far better solvent than absolute chloroform for morphine.

is evaporated on a water-bath or steam-bath, dried with dried sodium sulphate and powdered. The treatment of this powder with a portion of the first chloroformic extract involves a considerable loss of the latter through retention by the powder. It is necessary, therefore, to determine the relative amount of active principle removed in this part of the process and its nature, in order to learn whether it is desirable for the therapeutic actions of the preparation or for reasons of economy.

In order to determine the amount of the active principle removed from the powdered extract, we used a fresh portion of chloroform instead of an aliquot part of the first chloroformic extract, as directed in the official process. The results surprised us, and since our statement will almost certainly lead others to investigate this stage, we shall discuss our results in some detail.

Reference to Sluyters's paper shows that the first extraction with chloroform is designed chiefly to remove bigitalin and gitalin, the latter being partly decomposable in aqueous solution by heat, according to Kraft (4), and that the second part of the process (the evaporation of the aqueous residue and the extraction of the powder with chloroform) is intended to extract digitalein.

Sluyters prepared from the first aqueous residue a substance having an activity equal to 40 per cent of that of the first chloroformic extract, and he also found that the preparation made by the present official method is about 40 per cent more active than the first chloroformic extract alone. He convinced himself that digitalein is extracted by the second part of the process, but he says that the slight difference in yield hardly justifies the more troublesome process. We do not know just what Sluyters meant by digitalein, but he considered it different from the digitalein of Merck used by Hatcher in 1912 (5). Digitalein is available only in an impure form, and Cloetta (6) has shown that it consists of a mixture of active principles in combination with inert matter which renders it soluble in water.

Sluyters's statement concerning the activity of the second chloroformic extraction is directly opposed to our results and we must suppose that he failed to extract nearly so much of the active substance in the first treatment with chloroform as we did in every case. However, it is significant that Sluyters says that it hardly seems worth the trouble to extract the evaporated portion.

In our second experiment, we found that the activity of the first chloroformic residue was equal to that of a corresponding amount of finished digisol, indicating that the second chloroformic extraction was superfluous. We then prepared the second chloroformic extract with fresh chloroform in each of 12 experiments and in every case we found it inert. Each of us conducted the extraction independently but the results were alike.

There are several possible explanations of our failure to extract any active principle in this stage of the process: (1) the total removal of the chloroform-soluble substance in the first extraction; (2) destruction of the active principle by heat during evaporation; (3) conversion of the chloroform-soluble substance into a chloroform-insoluble substance by heat; (4) adsorption of the active principle by the sodium sulphate and inert residue, or other physical change.

(*1*) The first chloroformic extraction does not remove all the chloroform-soluble substance from the aqueous filtrate, and we found that when the first aqueous residue is filtered and then shaken with an equal volume of fresh chloro-

form, the second chloroformic extract contains from about one-fourteenth to about one-fifth as much active principle as the first, the yield depending in part on the skill with which the extraction is made. The second chloroformic residue prepared in this way usually contains a higher percentage of impurity than the first, and it is retained more tenaciously.

In an experiment of December 31, 1928, 200 Gm. of a mixture of five specimens of digitalis was used for the preparation of the first chloroformic extract in the usual manner, except that the aqueous filtrate was shaken with chloroform during two hours. The purified residue obtained after the distillation of 990 cc. of the first chloroformic extract, representing 123.75 Gm. digitalis, weighed 350.0 mg., the test showed that 1.27 mg. equalled 1 cat unit.

The aqueous residue was then divided into two equal portions of 500 cc. each. The first was evaporated in the usual way, 60 Gm. of dried sodium sulphate was added and the mixture was dried and powdered. The powder was treated with 500 cc. fresh chloroform during 24 hours; 450 cc. of this second chloroformic extract was filtered and distilled. The residue weighed 13.4 mg. and when tested on a cat it was found to be inert. The second portion of 500 cc. of aqueous residue was shaken with 500 cc. of fresh chloroform, during two hours. Four hundred seventy cc. of this second chloroformic extract was filtered and distilled. The residue weighed 57.7 mg. and after purification it weighed 37.7 mg. The test showed that 1.47 mg. equalled 1 cat unit. The total activity of the purified residue, representing the second chloroformic extract of 58.75 Gm. of digitalis, was equal to only 26 cat units, while the total activity of an amount of the first chloroformic extract representing an equal weight of digitalis was five times as great. The activity of a given weight of the purified second residue was about 80 per cent of that of an equal weight of the purified first residue. In an essentially similar experiment of February 28, 1929, the total activity of the purified first chloroformic residue was about 14 times that of the second chloroformic residue. This shows that the second chloroformic extraction of the aqueous residue even without evaporation is of doubtful economy because, while the action of the second chloroformic extract is qualitatively like that of the first, the total yield does not justify the labor involved, and we have found that the second chloroformic residue is more difficult to purify than the first.

(*2*) The following shows that the heat used in evaporating the aqueous residue does not destroy the active chloroform-soluble substance, and (*3*) that it does not convert it into a chloroform-insoluble substance. In one experiment, the powder left after the second chloroformic extraction with fresh chloroform was heated to expel the chloroform, the powder was then redissolved in sufficient water to make the volume equal to that of the original aqueous residue and this was shaken with an equal volume of chloroform. The latter extracted a little more of the active substance than was extracted by shaking an equal volume of the unevaporated first aqueous residue with an equal volume of fresh chloroform. This suggested that the addition of sodium sulphate to the aqueous filtrate facilitates the extraction with chloroform. We therefore performed the following experiment.

A fresh aqueous filtrate (which had not been extracted with chloroform) was evaporated and dried sodium sulphate was added in the manner directed for the

preparation of the second chloroformic extract. The powder was treated with fresh chloroform and while the resulting extract was not quite inert, its activity was negligible. The powder was then heated to expel the chloroform and it was dissolved in sufficient water to make it up to the original volume. This was shaken with an equal volume of chloroform, and the resulting chloroformic extract yielded a greater amount of residue than that which we had previously obtained by shaking an equal volume of the original filtrate with chloroform. This shows that the addition of sodium sulphate increased the yield of active principle, and that the heat used in evaporation did not destroy it. Subsequent tests confirmed this, but the difference is only about 12 per cent, hence it does not seem worth while to add the sodium sulphate. A greater amount of impurity was also extracted by the chloroform.

(*4*) The results just described point to adsorption of the active principle or to other physical changes whereby they are rendered insoluble in the dry state. If such a change does occur, it is reversible, as we have shown.

While the official second chloroformic extraction is superfluous, there are several minor points that should be discussed. The extract remaining after the evaporation of the first aqueous residue contains much hygroscopic matter and we have found it necessary to add about 10 Gm. of dried sodium sulphate for every 100 cc. of infusion evaporated. The resulting mass clings to the evaporating dish, and it is hygroscopic and tedious to reduce to a fine powder. The extraction involves the loss of about 12 per cent of the first chloroformic extract, which is retained by the powder. It is tedious to dissolve the second chloroformic residue in water according to the official directions, but the use of a volume of alcohol equal to about 1 per cent of the finished digisol, facilitates this step greatly.

Sluyters reports that the activity of his preparation was equal to about 49 per cent of the total activity of the leaf. We have never succeeded in preparing digisol with an activity greater than about 25 per cent of the total activity of the leaf, and our results are in harmony with those of others who have reported their results to us directly.

d. Temperature of Liquids during Extraction.—The results of Kraft's work and that of Cloetta inclined us to believe that it is unnecessary to maintain a temperature lower than 30° in the liquids during the extraction, and that the directions in the Netherlands Pharmacopœia to avoid a temperature above 15° involves unnecessary inconvenience. Experiments with a single species of digitalis are not absolutely conclusive but limitations of time have prevented our investigating this question with more than one specimen, and we are the more content to limit our study of the question because we know of no evidence to support the view that the inconveniently low temperature is of the slightest advantage.

In this experiment the aqueous filtrate was divided into two portions. The first portion was allowed to stand about 20 hours at a temperature varying from 28° to 23°, after which the purified residue was prepared in the usual manner and tested on cats. One hundred cc. of the chloroformic filtrate yielded 87 mg. of residue and 57 mg. of purified residue, of which 1.25 mg. equals 1 cat unit, this being the activity of the greater number of purified residues. The remainder of the aqueous filtrate was kept cold and the purified residue from it was prepared in the usual manner. One hundred cc. of chloroformic filtrate yielded 74.3 mg.

of residue and 52 mg. of purified residue. The yield of the purified residues and their activity indicate that no decomposition occurs at a temperature of 28° or lower. The fact that extraction at the higher temperature yielded slightly more of the unpurified residue and of the purified than that at the lower is of minor importance. It is possible that the extraction was a little more efficient, though an effort was made to shake both portions equally.

e. Purification of the Residue of the Chloroformic Extract.—One must know something of the nature of the active principles of digitalis and their solubilities in alcohol, in water and in chloroform, in order to understand the problem involved in the purification of a substance which is designed for injection or even for oral administration. The subject is extremely complex and space does not permit of our discussing it exhaustively. The following brief discussion is based partly on personal experience, partly on the work of Kraft (4), but mainly on that of Cloetta (6).

Cloetta believes that only three active principles of digitalis—crystalline digitoxin, crystalline bigitalin and crystalline gitalin—require consideration, though there are negligible amounts of other active substances present in the leaf. He also believes that digitoxin is the chief active principle in digitalis. The pure crystalline digitoxin is insoluble in water but soluble in dilute alcohol and readily soluble in chloroform. Pure crystalline bigitalin is characterized by its slight solubility in available solvents including water, alcohol and chloroform; pure crystalline gitalin is slightly soluble in water, readily soluble in alcohol and in chloroform. All of these substances are extracted from the leaf by water in physical combination with various impurities from which they are separated with difficulty and only with great loss, the yield of pure principle in any case being only a fraction of that existing in the leaf. While it is usually preferable to employ pure principles in therapeutics, convenience and economy dictate the use of impure principles of digitalis in the majority of cases, but it is desirable that any preparation of digitalis that is injected shall contain no more of the impurity than is necessary to hold the active principles in solution.

Simple inspection shows that the digisol of the Netherlands Pharmacopœia contains an odoriferous principle (volatile oil) and coloring matter (chlorophyl), and the fact that it froths when shaken shows the presence of saponin. Mixtures of chloroform, ether and petroleum ether have often been recommended for purifying preparations of digitalis and for the estimation of Keller's so-called digitoxin. We have used this simple procedure in the purification of the first chloroformic residues, and have usually obtained a purified residue of which about 1.25 mg. equals 1 cat unit.

We have not determined the minimum amounts of chloroform, ether and petroleum ether required for the purification of the chloroformic residue and it is almost certain that different amounts will be required with the chloroformic residues of different specimens of digitalis in order to produce a residue of this degree of activity.

In one experiment the purification of 100 mg. of residue by dissolving it in 1 Gm. of chloroform and adding $2^1/_2$ Gm. of ether and $17^1/_2$ Gm. of petroleum ether appeared satisfactory, but in an experiment of February 26, 1929, 308.7 mg. of slightly atypical (sticky) residue was dissolved in 3 Gm. of chloroform and to this was added 7.5 Gm. of ether and 52.5 Gm. of petroleum ether. The pre-

cipitate weighed 226.7 mg. after the removal of 82.0 mg., or 26.6 per cent, of soluble inert substance. The precipitate was redissolved in 8 Gm. of chloroform and to this was added 20 Gm. of ether and 140 Gm. of petroleum ether. The dried precipitate weighed 170 mg., after the removal of 56.7 mg., or 25 per cent, of soluble inert matter.

In the experiment of February 28, 1929, 705 cc. of the first chloroformic extract, representing 88 Gm. of digitalis, yielded 395.8 mg. of residue. This was dissolved in 4 Gm. of chloroform and to it was added 10 Gm. of ether and 70 Gm. of petroleum ether. The soluble inert impurity weighed 118.3 mg.; the active precipitate weighed 277.5 mg.; this was redissolved in 8 Gm. of chloroform and to it was added 20 Gm. of ether and 140 Gm. of petroleum ether. There remained in solution 30.5 mg. of inert impurity, leaving 247.0 mg. of active residue. The first aqueous residue was then treated with 704 cc. of fresh chloroform, the chloroform was distilled leaving 110 mg. of residue. This second chloroformic residue was dissolved in 4 Gm. of chloroform and to it was added 10 Gm. of ether and 70 Gm. of petroleum ether. Sixty-five milligrams of inert matter remained in solution; the purified residue weighed 45 mg. The test showed that 3 mg. of this purified residue equalled 1 cat unit. Hence, it was only 40 per cent as active as an equal weight of the average purified first chloroformic residue, and the total second fresh chloroformic purified residue from 88 Gm. of digitalis had a total activity of only 15 cat units, or about one-fourteenth as great as that of the purified residue of the first chloroformic extract.

Briefly, 1 Gm. of chloroform, 2.5 Gm. of ether and 17.5 Gm. of petroleum ether for each 100 mg. of the first chloroformic residue sufficed for the removal of 118.3 mg. of inert matter, equal to 30 per cent of the weight of the residue, after which twice as much chloroform, ether and petroleum ether removed only about one-fourth as much inert matter. A much larger relative amount of chloroform, ether and petroleum ether removed a larger percentage of inert matter, from the second chloroformic residue, but the purified residue was then less active than that from the first chloroformic extract, that is, it contained a greater percentage of impurity.

In some experiments, we used 4 Gm. of chloroform, 10 Gm. of ether and 70 Gm. of petroleum ether for about 100 mg. of chloroformic residue. The impurity remaining in solution in this mixture was tested in a number of experiments and in every case was found to be inert, but when a smaller proportion of petroleum ether was used, part of the active principles was removed with the impurity. Our results indicate that a larger proportion of chloroform, ether and petroleum ether is necessary for the purification of some extracts than for others but we tentatively recommend the use of 2 Gm. of chloroform, 5 Gm. of ether and 35 Gm. of petroleum ether for 100 mg. of first chloroformic residue.

Cloetta (6) states that digitalis does not contain any active principle which in a pure state is readily soluble in water. He states that pure gitalin is about one-third to one-half as active as pure digitoxin for cats by subcutaneous injection, and for frogs. The purified first chloroformic residues that we prepared were nearly uniformly about one-fourth as active when injected intravenously in cats as Merck's digitoxin, which, however, is not absolutely pure, according to Kraft. At any rate, the unpurified first chloroformic residue is probably about as pure as

it is desirable to have it for intramuscular injection because products of much greater purity are not sufficiently soluble in water for convenient intramuscular injection.

f. Active Constituents of Digisol.—It may seem to the reader that it is incumbent upon an investigator to state the composition of any preparation that he discusses in terms that are in common use or in terms of pure principles of a recognized authority. Unfortunately, it is not possible for us to do this with reference to digisol with exactness though one of us is studying the literature in the effort to learn the relationship of the various so-called digitalis principles in common use and the more or less pure principles that have been described by Nativelle (7), Schmiedeberg (8), Kiliani (9), Kraft (4), Cloetta (6) and others.

Many impure substances prepared from digitalis by different investigators have become established in the literature and in common use under names that were given them by their discoverers who supposed them to be pure principles, but which have been found to be mixtures of various active principles with impurities. Some of these substances, such as crystalline digitaline, or digitoxin, as it is commonly called, consist of nearly pure principles. German digitalin, so-called, is said to consist of about sixty per cent of digitonin and it is probably of very variable composition. The confusion is increased by the fact that similar or nearly identical names have been given to wholly different substances. The general reader is referred to "New and Nonofficial Remedies" (1928), 143–154, published by the American Medical Association, Chicago, for a brief discussion of digitalis principles and preparations.

Special reference has been made to the recent work of Kraft and to that of Cloetta. Some of Kraft's claims have been disproved and it is impossible to state to what extent further investigation will confirm or disprove the main points in Cloetta's work, but at this time it appears to be of fundamental importance, and it is desirable to state the approximate composition of digisol in terms of the active principles described by Cloetta—crystalline digitoxin, crystalline gitalin and crystalline bigitalin, and of digitalin, digitalein and the gitalin of Kraft.

Kraft maintains that cold water does not extract digitoxin from digitalis, but Cloetta found that it constitutes more than one-third of the active principles of the watery extract, and since it is soluble in chloroform, we may be certain that at least a small amount is present in digisol. The remainder of the active substance in digisol consists of crystalline gitalin and crystalline bigitalin and their genins.

Dooley (10), working in this laboratory, showed that tincture of digitalis contains a principle that is eliminated rapidly after its intravenous injection in the cat, and Weiss and Hatcher (11) showed that a rapidly eliminated substance is present in tincture of digitalis, in chloroformic extracts of the infusion prepared with hot water, in ethereal extracts of the preceding, in so-called digitoxin Keller, in a chloroformic percolate of digitalis, in a commercial specimen labelled digitoxin, and in a specimen of crude digitalein. There is little doubt that this rapidly eliminated substance consists of genins of the gitalin group—gitaligenin and bigitaligenin—not of digitoxigenin, which is eliminated slowly.

Weiss and Hatcher prepared a chloroformic extract similar to the first digisol chloroformic extract. They shook a one per cent cold infusion of digitalis with a

nearly equal volume of chloroform. Their first chloroformic residue was identical with that prepared by us in the present investigation, and they found that it contained very little of the rapidly eliminated fraction. They evaporated the infusion left after the first chloroformic extraction, precipitated the inert matter with alcohol, dissolved the residue in water and shook it with chloroform. They obtained about 2 mg. of residue for each gram of digitalis. It consisted almost wholly of the rapidly eliminated fraction, and cats eliminated nearly fatal doses within periods of about three hours.

The results reported by Weiss and Hatcher made it appear almost superfluous to examine digisol for the presence of the rapidly eliminated substance. Nevertheless we have examined it. The method employed is similar to that used by Weiss and Hatcher. It consists in determining the difference between the fatal dose when the drug is injected rapidly and that when it is injected slowly into a vein of the cat. No difference could be detected between the dose required to cause death when the injection was completed within about 30 minutes and that when it was completed in about three hours. This shows that digisol does not contain more than a very small proportion of rapidly eliminated substance (genins of the gitalin group).

The average fatal dose of a purified residue was 1.11 mg. per Kg. when the injection was made in an average of 1 hour and 46 minutes, in a series of 3 experiments, and the average dose was 1.15 mg. per Kg. when the average period of injection was 3 hours and 17 minutes in another series of 3 experiments. While this indicates that the active substance contains a small percentage of the rapidly eliminated principles, the difference is within the limits of error. In fact, it seems probable that small amounts of the rapidly eliminated genins are present, and since Weiss and Hatcher found little else in the second chloroformic extract of their evaporated aqueous residue, it seems certain that the second chloroformic extract of digisol would contain them if the method used permitted of their extraction. It is possible that Sluyters did actually extract these genins, but they add nothing of value to the preparation and it is better without them.

One gram of digitalis of good quality, of which about 100 mg. equals 1 cat unit, usually yields about 2.75 mg. of purified residue, equal to 2.2 cat units, hence, the purified residue represents about 22 per cent of the total activity of the leaf. When injected intravenously into cats, it is nearly as active as Kraft's gitalin, about half as active as crystalline gitalin, and about one-fourth as active as Merck's crystalline digitoxin.

It gives the Keller reaction, a blue layer at the point of contact, the acetic solution gradually becoming blue-green when a little of the purified residue is dissolved in glacial acetic acid, a trace of ferric chloride is added and the solution is allowed to overlie sulphuric acid or Kiliani's reagent without mixing,[1] it also gives a red ring at the zone of contact of the acids. The color test is of little significance, however, since the blue-green is due to digitoxose, according to Cloetta, and is given by gitalin, bigitalin or digitoxin, while the red ring is given by free genins. The purified residue of digisol with Kiliani's reagent gives a cherry-red color.

[1] Kiliani's reagent consists of 0.05 Gm. ferrous sulphate, dissolved in 1 cc. of water and added to 100 cc. of pure sulphuric acid.

From this brief discussion and all the facts available, we may say that the purified chloroformic residue consists mainly of gitalin with small amounts of bigitalin, digitoxin and free genins of the gitalin group, together with impurities, including a trace of saponin and substances of unknown character which serve to render the active principles soluble in water and in dilute alcohol. It is related to the digitalein of commerce but that substance is so variable that it has ceased to have a definite meaning. True digitalin, the digitalinum verum of Kiliani, whatever it may be, differs widely from digitoxin and it almost certainly plays no essential rôle in the action of digitalis, and this is true in an even greater degree of the so-called German digitalin, hence they require no further consideration here.

The active substance of digisol is identical with a chloroformic residue prepared by Weiss and Hatcher in 1921, to which reference has already been made. They did not seek to introduce it because they were not convinced that it is superior to ouabain for intravenous injection or to a good specimen of tincture of digitalis for oral administration, and, on the other hand, it is more expensive than tincture of digitalis. Digisol or the purified chloroformic residue differs from the official infusion and tincture of digitalis, both of which represent practically all the activity of the leaf, despite the statement of numerous investigators that digitoxin is insoluble in water. Since it represents only about 20 per cent of the total activity of the leaf, it does not contain all of the gitalin and bigitalin or else we must suppose that digitalis contains other active substances in addition to the digitoxin, gitalin and bigitalin mentioned by Cloetta. The unpurified residue is probably as nearly pure as any active principle from digitalis can be, while retaining a sufficient degree of solubility in water or in normal salt solution to permit of its convenient use for intramuscular injection. The purified residue may be used for oral administration for which purpose it may be dissolved in a small amount of alcohol and diluted with water.

The solubilities of each of the active principles of digitalis are influenced by the presence of the other active substances, and, more especially, by impurities of unknown composition, and the intensity of the effect varies with the relative amounts, hence there is almost no limit to the number of impure substances of different solubilities that may be prepared from digitalis.

g. Stability of Digisol and of the Purified Residue.—The stability of various preparations of digitalis has been the subject of as much dispute as various other problems concerned with this drug. It has been stated that infusion of digitalis decomposes with such rapidity that it is of little value after a single day. On the other hand, Hatcher and Eggleston (12) found that infusion of digitalis decomposes very slowly. Various investigators have reported the rapid or slow deterioration of tinctures of digitalis, but one of us has maintained that almost every tincture of digitalis prepared with a high percentage of alcohol keeps almost indefinitely, though he has seen one specimen of the tincture prepared by himself lose half of its activity within a year.[1] There is also a wide diversity of opinion concerning the readiness with which various digitalis principles and preparations are destroyed in the gastro-intestinal tract. We have therefore conducted ex-

[1] Work now in progress in this laboratory, indicates that sterile infusions of digitalis and tinctures undergo slow deterioration during many years.

periments to show (1) the stability of digisol in vitro and (2) that of the purified residue in diluted hydrochloric acid.

(*1*) *Stability of Digisol Kept in Glass.*—A specimen of digisol made according to the directions of the Netherlands Pharmacopœia on September 4, 1928, except that it was somewhat more dilute than directed, after sterilization was placed in nearly filled bottles each holding 60 cc., these were sealed hermetically and kept in the laboratory during a period of six months, after which the activity was tested on cats in the usual manner. This solution, when tested on September 17, 1928, required an average of 5.26 cc. per cat unit in an average of 2 hours and 32 minutes. When tested on March 8, 1929, it required an average of 5.98 cc. in an average of 2 hours and 45 minutes. Hence, the activity after an interval of 6 months was 88 per cent of that found shortly after its preparation. This is within the limits of possible error and one cannot be absolutely certain that the specimen had undergone any deterioration. In the absence of further evidence, it would seem that digisol should not be kept more than one year.

(*2*) *Stability of the Purified Residue in 0.2 Per Cent Hydrochloric Acid.*—Cloetta states that crystalline gitalin is decomposed readily by dilute hydrochloric acid in the cold, with the liberation of gitaligenin which has a much weaker action than gitalin, whereas bigitalin is very much more stable and requires heat or a much stronger concentration of hydrochloric acid for its rapid decomposition.

Thirty milligrams of a specimen of purified chloroformic residue was dissolved in a few drops of alcohol and to this was added 45 cc. of 0.2 per cent of hydrochloric acid in which it was not perfectly soluble, though no actual precipitate formed. The solution was maintained at a temperature of 37° during two hours, after which about 10 cc. of alcohol was added and the acid was neutralized by the addition of 29 cc. of $N/10$ sodium hydrate, forming an almost perfectly clear solution. Enough water was added with sodium chloride to make the total measure 375 cc. of 0.85 per cent sodium chloride solution with about 3 per cent of alcohol. The solution would have contained one part of residue, had none been destroyed, in 12,500 of solution. This was then tested on 3 cats in the usual manner.

The activity of this specimen of purified residue, when diluted with 12,500 parts of 3 per cent alcohol in normal salt solution was such that 1.33 mg. equalled 1 cat unit. The test indicated that more than half of the active substance had been destroyed by the hydrochloric acid. One cat unit then corresponded to the undecomposed portion of 2.84 mg., that is, the average dose required was 35.5 cc. of the solution per Kg. of weight. Treatment with weak hydrochloric acid liberates genins of low activity, but we made no effort to separate these from the solution.

It is probable that no specimen of digisol administered orally to man would ever be in contact with 0.2 per cent of free hydrochloric acid during two hours. Nevertheless, the results of this experiment, which are in harmony with the statement of Cloetta, indicate that if digisol or the purified chloroformic residue is administered orally, it should be given while the patient is fasting.

This experiment also affords additional evidence that digisol and the purified chloroformic residue owe their activity mainly to gitalin, since bigitalin is not decomposed readily by such dilute hydrochloric acid without heat, and there is other evidence that they contain little digitoxin.

(*To be continued*)