A PREPARATION OF DIGITALIS SUITABLE FOR INJECTION OR ORAL ADMINISTRATION.

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B. Method of Preparation Recommended.—Should it be decided to admit a preparation of the type of digisol to the U. S. P. XI, further experimentation will be necessary, and we offer the following merely as a basis for consideration, because it possesses distinct advantages over the method of the Netherlands Pharmacopœia V.

Add 1000 cc. of cold distilled water to 125 Gm. of digitalis in No. 30 powder, maintain a temperature of 25° or lower, and shake frequently during 24 hours. Filter, shake the filtrate with an equal volume of chloroform in a suitable vessel during a period of 2 hours, avoiding excessive emulsification. Collect the chloroformic layer, pass it through a hard filter, previously wetted with chloroform, distill the chloroformic filtrate to a small volume and allow the remainder of the chloroform to evaporate spontaneously. Dissolve 200 mg. of the residue in 4 Gm. of chloroform, add 75 Gm. of petroleum ether in small portions so as to obtain a flocculent precipitate, allow the mixture to stand an hour or longer, collect the precipitate and dissolve it in 20 cc. of alcohol, add enough sterile water to make 100 cc., standardize the solution, and adjust the volume so that 2 cc. will equal 1 cat unit, filter if necessary, and sterilize the solution.

When the solution is designed for oral administration, the residue may be purified as follows: Dissolve 200 mg. of the residue in 4 Gm. of chloroform in an Erlenmeyer flask, add 10 Gm. of ether and 70 Gm. of petroleum ether; cork the flask tightly and allow it to stand in a cool place several hours or over night. Collect the precipitate, dissolve it in 20 cc. of alcohol, add enough water to make the solution measure 100 cc. Standardize the solution and adjust the volume so that 2 cc. will equal 1 cat unit.

Objection may be made to the recommendation concerning the standardization, but since we do not know the ratio of activity of digisol to that of ouabain by the frog method, we cannot recommend the frog method at present. Famulener and Lyons found amorphous strophanthin 17 times as active as digitoxin by the frog method. The daily intravenous dose of amorphous strophanthin is 0.5 mg. (U. S. P. X) but the first injection of 17 times that amount of digitoxin would almost certainly cause death at once. The frogs commonly used in this laboratory are obtained from St. Albans, Vermont, and usually we have not found them well suited for the standardization of digitalis. We do not mean to imply that others should not determine the therapeutic dose based on an assay on frogs.

Sluyters says that digisol of the Netherlands Pharmacopœia may be made by the retail pharmacist. This may be possible, but there are comparatively few pharmacists who are sufficiently trained in biologic assay methods to undertake the standardization of digisol. If this were the only preparation to be assayed biologically the technic might offer no serious obstacle, but with the constantly increasing requirements of biological assay methods, the pharmacist would require a special department for this work alone. That is manifestly impossible for more than relatively few retail pharmacists in the United States.

Unfortunately, physicians are prejudiced against ouabain and strophanthin because of deaths that have followed their unwise use, and they continue to employ widely advertised proprietary preparations because they find encouragement and fancied security in following the directions of the manufacturer, and there is a practical need of an official preparation of digitalis suitable for intravenous and intramuscular injection.

The method of preparation of digisol of the Netherlands Pharmacopœia will undergo changes with criticism that will develop, hence confusion will be avoided by the use of a distinctive name for any preparation of this type that will be admitted to the U. S. P. XI.

II.   EMETIC ACTIVITY.

All digitalis principles and preparations cause vomiting when the full therapeutic dose is exceeded slightly, and Eggleston and Hatcher (13) found the average emetic intravenous dose to vary from 22 per cent of the fatal with true digitalin to 65 per cent of the fatal with amorphous strophanthin. The average emetic dose of the specimen of the digitalis that they used was 46 per cent of the fatal; that of ouabain was 49 per cent, and that of crystalline digitoxin was 58 per cent of the fatal. They found digipuratum, digitalysatum and digalen relatively more actively emetic in proportion to their toxicity than digitalis.

We have therefore determined the emetic activity of the first chloroformic residue. One cat vomited 5 minutes after the intravenous injection of one-third of the average fatal dose, and three times at short intervals thereafter. Another cat failed to vomit during a half hour following the intravenous injection of one-fourth of the average fatal dose but it vomited four minutes after the intravenous injection of an additional 5 per cent of the fatal dose, or a total of 30 per cent of the fatal dose. Vomiting was repeated after one minute and again after three minutes, this dose being obviously near the minimum. One cat showed nausea within five minutes after the intravenous injection of one-half of the fatal vein dose, it vomited after two minutes and three times at short intervals thereafter. All three cats appeared depressed and all died after varying periods. One died 5 days after a dose equal to one-half the average fatal dose (as determined in the usual manner); the other two died, one after an interval of 7 days, the other after an interval of 14 days. This unexpected result caused us to repeat the experiment with the same specimen of purified residue. The emetic effects were closely similar to those observed in the first series but none of the three cats died. This is in harmony with the results in numerous experiments on persistence of action in which only 3 cats died after the injection of 75 or 80 per cent of the estimated fatal dose, until killed by the injection of ouabain after intervals of 1 to 5 days.

III.   ABSORBABILITY.

a.   Rate of Absorption from Muscle.—Different drugs are absorbed from muscle at widely different rates dependent upon many factors. Hatcher and Gold (14) recovered morphine sulphate from the muscle at the seat of injection in amounts up to 42 per cent of that injected after intervals of 30 minutes, and many soluble drugs are absorbed even more slowly. An interval of two hours sometimes follows the intramuscular injection of an amount of ouabain equal to twice the average fatal dose before death results.

We have used the onset of emesis and death to estimate the rate of absorption of the first chloroformic residue after the intramuscular injection of amounts equal to twice the average fatal dose. The intravenous injection of an amount equal to about one-sixth of that dose causes emesis, and the injection of one-half of the dose causes death. The results indicated that one-sixth of the total intramuscular dose was absorbed in 23 minutes, in 24 minutes and in 48 minutes, respectively, in three experiments, and that one-half of the total intramuscular dose was absorbed in 2 hours 39 minutes in one case, in 3 hours 38 minutes in another and in more than 9 hours in a third. The third animal was probably tolerant, and the rate of absorption in the other two until death was not widely different from that until the onset of vomiting, and from these experiments we concluded that roughly one-sixth of the intramuscular dose is absorbed in an average of one-half hour but that the rate of absorption declines gradually and that one-half of the intramuscular dose is absorbed within about 3 hours. The figures are not exact and the rate of distribution in the body after intramuscular injection is almost certainly somewhat different from that after intravenous injection, because different tissues fix poisons and remove them from the blood stream with different degrees of effectiveness. For example, Weiss and Hatcher (15) found that the kidneys continue to remove strychnine and to eliminate it in the urine when only the merest traces are present in the circulation.

b. *Rate of Absorption from the Gastro-Intestinal Tract.*—No digitalis body is absorbed at a uniform rate from the gastro-intestinal tract and a good many drugs of this group are absorbed so slowly and so irregularly that they are not suited for oral therapeutic use. Marvin and White (16) found that massive doses of convallaria or apocynum are required to maintain the digitalis action in man, and workers in this laboratory have long held that strophanthus is unsuited for oral administration because of the extreme irregularity in the rate of its absorption from the gastro-intestinal tract. We have sought to determine the approximate rate of absorption of digisol from the gastro-intestinal tract of the cat because one of us has long been interested in the problem of preparing a substitute for tincture of digitalis that would be absorbed from the gastro-intestinal tract at a more nearly uniform rate than is the tincture or the infusion.

The absorption of a chloroform-soluble substance, closely similar to digisol, was studied by Hatcher (17) and by Eggleston and Wyckoff (18) who said it is absorbed from the human digestive tract more rapidly and more nearly uniformly than are different specimens of official tincture of average biologic activity.

The results of our experiments indicate that digisol is absorbed about as well as tincture of digitalis, and they are in harmony with the observations of Hatcher, and those of Eggleston and Wyckoff just mentioned, hence they do not call for detailed consideration.

c. *Interval before Action after Intravenous Injection.*—The action on the heart begins within a few seconds after the intravenous injection of a large dose of digitoxin, and within two minutes after the injection of a large dose of ouabain, and the action of therapeutic doses often begins within a few minutes after intravenous injection, though the full effects may be delayed many hours. We therefore thought it worth while to determine whether the purified residue acts in this respect like digitoxin or only after a considerable delay.

A dose of 2.5 mg. of purified first chloroformic residue per Kg. of weight, equal to twice the average fatal vein dose, in 5 cc. of 16 per cent alcohol in normal salt solution was injected intravenously into a cat in a period of 22 seconds, while a tracing was being taken to show the carotid blood pressure and pulse rate.[1]

There was a gradual increase in the blood pressure beginning seven seconds after starting the injection; it reached 250 millimeters of mercury, after which it returned gradually to the previous level in 2 minutes and 15 seconds. The rate became slower immediately after the injection was completed, and 15 seconds later the slowing was marked and was attended with irregularities. There was a gradual fall of blood pressure to 100 mm., and after 32 minutes, an additional dose of 1.25 mg. of the residue per Kg. equal to the average fatal dose, was injected in 13 seconds. The heart stopped one minute later. In another nearly similar experiment, the results were nearly the same. In a third experiment, 5 mg. of the residue per Kg., in 18.4 cc. of 10 per cent alcohol (equal to 4 times the average fatal dose) was injected in a period of 19 seconds. The rate became slower during the injection, and after 80 seconds irregularities were observed which persisted until death, which occurred 5 minutes and 20 seconds after beginning the injection.

This behavior of the purified residue recalls that of digitoxin with reference to the prompt onset of action, but it resembles ouabain with reference to the delay before death occurs. It presents one rather striking contrast to the behavior of pure digitoxin, a massive dose of which produces death within a few seconds.

We believe that digisol or a solution of the first chloroformic residue would prove satisfactory in clinical use, but physicians must learn the technic of employing it, otherwise there will be disappointment following the use of ineffective doses, or accidents following the use of too much. This is true of every preparation, but it has not prevented the introduction of many proprietary preparations which have no obvious advantage over the tincture and the infusion, but which sell at prices many times greater than equivalent amounts of the official preparation, and the advantages of having an official non-secret preparation suitable for injection are obvious.

IV. PERSISTENCE OF ACTION.

The various active principles of digitalis exist in the leaf in physical combinations which undergo changes during extraction with water and under a variety of conditions, hence, a relatively slight difference in the method of preparation may cause a considerable difference in the relative amounts of the active principles extracted, and in the physical combinations in which they exist in the preparation. The physical state in which any one of the digitalis glucosides is combined exerts a marked influence on its solubility and on its absorbability from tissues, and this in turn modifies its activity.

Hatcher (5) found that the different digitalis bodies vary widely in the length of time during which the action persists in the cat, and Eggleston (19) found that the persistence of action of most of the digitalis bodies in man is closely similar to that found by Hatcher in the cat. A relatively high percentage of digitoxin

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[1] The injection of a similiar dose of alcohol in normal salt solution produced practically no change in the blood pressure or in the heart rate.

causes long lasting action, whereas a relatively high percentage of gitalin and bigitalin or their genins induces a much less persistent action. We have therefore investigated the persistence of action of the first chloroformic residue by the method used by Hatcher.

The method, in brief, is as follows: The animal receives an intravenous injection of a known percentage of the average fatal dose of the specimen to be examined and after the required interval following this initial dose, the amount of ouabain required to cause death is determined. The difference between the dose of ouabain required at that time and the average full fatal dose—0.1 mg. per Kg.— is due to that part of the action of the initial dose of the substance to be tested, which has persisted. If an animal requires 0.05 mg. of ouabain per Kg., or 50 per cent of the average fatal dose, after an interval of two days following the injection of an initial dose of 80 per cent of the average fatal dose of the specimen under examination (chloroformic residue), we assume that the action of 50 per cent of the fatal dose, or 62.5 per cent of the initial dose, persists. While the figures are given in accordance with the exact calculations, it must be understood that they are only approximately correct.

Gold (20) observed that in cats the persistence of action determined 24 hours after the administration of an initial dose of digitalis was less than that after an interval of 48 hours. We have confirmed this seemingly paradoxical statement and have found a greater persistence of action on the third day after the initial .dose, and even on the fifth, than after an interval of 24 hours. This can mean nothing less than that the effect—not necessarily the direct cardiac action—increases during more than 24 hours in the cat. Cloetta (6) believes that digitoxin is decomposed in the body with the slow liberation of digitoxigenin, and that digitoxigenin is about one-half as active as digitoxin. He attributes the cumulation action (which is nothing but persistence of action or increased effect) to this fact, and it is possible that the slow liberation of digitoxigenin from digitoxin fixed in tissues outside of the heart is partly responsible for the increasing action after an interval of 24 hours. Cloetta's theory is based on pure assumption, and there is no proof that digitoxigenin is liberated in the animal body or that it is responsible for the so-called cumulative effects of digitoxin and digitalis, and, on the other hand, so-called cumulation is seen with other digitalis bodies and which do not yield digitoxigenin.

We have not attempted to determine the exact duration of the persistence of action of digisol or that of the first chloroformic residue (and one may be sure that it varies rather widely in different animals), but two series of experiments were conducted to show the persistence of action. In the first series each of 8 cats received an intravenous injection of 80 per cent of the average fatal dose of the first chloroformic residue of the experiment of December 31, 1928, which was prepared from a mixture of 5 specimens of digitalis. The results show that the average action persisting was equal to 58.4 per cent of the initial dose after an interval of 2 days; to 77.8 per cent after 4 days, and to 69.6 per cent after 5 days. In the second series each of 15 cats received an intravenous injection of 75 per cent of the average fatal dose of the chloroformic residue obtained from the experiment of January 10, 1929. In the second series the action persisting after 24 hours was equal to that of 62.4 per cent of the initial dose, and to 88.4 per cent after 2 days;

one of those tested after three days was obviously tolerant, and three others tested at the same time showed an average of 56.9 per cent of persistence of action. The average in two animals tested after 4 days was 62.5 per cent; and that of two after an interval of 5 days was 61.4 per cent.

It is probable that the persistence of action that is due to gitalin and bigitalin diminishes rapidly, and that that due to digitoxin diminishes more slowly, but one may assume that much the greater part of the action disappears within ten days.

V.   SUMMARY AND CONCLUSIONS.

1. The theoretical basis of the method employed in the extraction of the Netherlands Pharmacopœia V is discussed and it appears that the first extraction with chloroform is designed mainly to extract gitalin, with some bigitalin and digitoxin, and that the second is designed to extract the hypothetical substance, digitalein.

2. No active principle is extracted by the treatment of the powder with chloroform in the second part of this process.

3. There is no pure principle called digitalein and we have discussed briefly the composition of commercial digitalein.

4. It is not necessary to extract the digitalis powder with water during more than 24 hours.

5. It is not necessary to shake the aqueous filtrate with chloroform during more than 2 hours in a capacious flask or other vessel. The emulsion formed separates on standing, or it may be separated almost completely into its components by shaking with an excess of fresh chloroform.

6. It is not necessary to maintain a temperature lower than 25° during extraction with water and with chloroform.

7. A method of preparation is offered as a basis for further study.

8. The weight of the residue of the first chloroformic residue and its relative activity vary with different specimens of digitalis and also with the method of drying, because it contains some volatile oil, part of which is expelled by heat.

9. The residue of the chloroformic extract may be purified by dissolving 200 mg. in 4 Gm. of chloroform, adding 10 Gm. of ether and 70 Gm. of petroleum ether and collecting the precipitate.

10. The purified residue obtained from different specimens of digitalis is of nearly constant activity, about 1.25 mg. being equal to 1 cat unit.

11. The chloroformic residue is more soluble in 10 per cent alcohol with distilled water than in normal salt solution to which 10 per cent alcohol has been added.

12. The solubilities of active principles vary with slight differences in the amounts of impurities present, and relatively slight differences in the method of procedure (temperature, etc.) result in notable differences in the amount of impurities present in the unpurified residue.

13. Digisol (or the first chloroformic residue) consists mainly of impure gitalin with small amounts of impure bigitalin, digitoxin and genins of the gitalin group, together with traces of saponin and other impurities of unknown composition.

14. Digisol dissolved in sterile normal salt solution and kept in tightly corked bottles loses little of its activity at room temperature within a period of six months.

15. The gitalin fraction of digisol or the purified residue is rapidly decomposed by 0.2 per cent hydrochloric acid at body temperature. The remaining fraction is more resistant to hydrochloric acid.

16. Digisol, or the first chloroformic residue, is a little more actively emetic than the tincture of digitalis U. S. P. in proportion to the toxicity shown by standardization on cats.

17. The rate of absorption from the gastro-intestinal tract is comparable to that of tincture of digitalis. The rate of absorption from the muscle is not sufficiently rapid to point to the use of the purified residue or digisol by intramuscular injection in emergencies requiring immediate therapeutic effects, but this is a crucial question (for this type of drug) and it requires critical clinical investigation.

18. The action following the intravenous injection begins at once but the full effects are not induced so rapidly as after a very large dose of digitoxin. The residue is well suited for intravenous injection in emergencies. The degree of purification to insure the optimum solubility requires further study.

19. The persistence of action is greater than that of strophanthin or ouabain, but apparently less than that of digitoxin or digitalis.

20. The distinction has been drawn constantly in this paper between the purified and the unpurified chloroformic residues, but the purified residue is only relatively pure, and the unpurified contains no impurity that is in the least injurious, but, on the contrary, the impurities present are of distinct advantage in increasing the solubility of the active principles in water.

21. The present investigation is only a preliminary study and numerous minor problems require further investigation before a preparation of the type of digisol is admitted to the Pharmacopœia.

CONCLUSIONS.

A chloroformic extract of a cold infusion of digitalis may be prepared of nearly uniform activity. It contains gitalin with small amounts of bigitalin, digitoxin and genins of the gitalin group, all in combination with useful impurities. A solution of the residue of this chloroformic extract, similar to digisol, is suitable for intramuscular or intravenous injection or for oral administration.

It is probably desirable that such a preparation be admitted to the Pharmacopœia, for reasons that have been discussed, though it has no demonstrable advantage over crystalline ouabain from a purely theoretical standpoint. It is essential that the therapeutic dose be established accurately before such a preparation is made official in order to avoid disappointment and accidents. A clinical investigation of the purified and the unpurified chloroformic residues is planned with a view to determining the dosage, including the necessary and permissible frequency of repetition of fractional doses based on observations of the persistence of action in man.

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A CONSIDERATION OF THE METHODS AND RESULTS IN THE STANDARDIZATION OF THE OVARIAN HORMONE.

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In view of the increasing interest in the estrus-producing principle and of the fact that the subject has been considered from various standpoints, it seems advisable to consider, in a collective way, or to analyze the present methods used in the physiological standardization of this remarkable substance. Prior to 1917 it was difficult by external observations to determine the stage of the estrus cycle of either rats, mice or guinea-pigs.

In 1912 Adler (1) stated that by injecting watery extracts of ovaries into animals he was able to produce all the symptoms of estrus. Later, Iscovesco (2), Fellner (3) and co-workers, and then Herman and Frankel (4) carried on this line of work.

In 1917 Stockard and Papanicolaou (5) showed that it was possible to follow the cycle in the guinea-pig by microscopical examination of the cells contained in the vaginal smear.

In 1922 Long and Evans (6) applied the principle to a study of the conditions in the rat. In the same year Allen (7) studied those in the mouse.

In 1923 Hartman (8) applied the same principle to the conditions in the opossum. This same year, Allen and Doisy (9) obtained a solution containing the active principle from the follicular fluid obtained from pig ovaries.

Parkes (10) applied the principle to the study of the conditions in the mouse in 1926.

There have been many other workers in this field notable among whom should be mentioned Laqueur (11) Dodds, Dickens (12) and co-workers, and Zondek and Ascheim (13).

As our experience with different products has advanced, naturally there have been developments in different laboratories which undoubtedly are an advantage. This, however, we feel would be lost unless these differences in technique and procedure are reconciled with one another. In other words, in order for the findings in this laboratory to be compared with those of another there must be some common ground upon which they stand.