A CRITICISM OF THE BIOLOGIC METHODS FOR THE STANDARDIZATION OF DIGITALIS WITH A SUGGESTION FOR A NEW METHOD.*

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I. INTRODUCTION. II. BIOLOGIC METHODS. III. CRITICISMS. IV. SUGGESTED METHOD. V. SUMMARY.

I. INTRODUCTION.

Since Withering in 1776 suggested the use of the purple foxglove as a diuretic, and Barton in 1798 determined that it slowed the action of the heart, this drug has been the subject of more investigation than any other of the Pharmacopœia. Strange to say, although volumes have been written, chemists, physiologists and pharmacologists the world over are still seeking some simple and accurate method for its standardization, and while we acknowledge that a great deal has been accomplished, we are nevertheless confronted with the fact that the preparations found upon the market to-day show as great a variance in toxicity when tested by biologic methods as they did when Pratt in 1910 announced, after the examination of a number of preparations tested by one of the frog methods, that he found a variation of as much as three hundred percent. Since this statement a number of careful investigators have reported the startling fact that we may have preparations of this drug that are either too active or worthless. When we pause and consider the importance of this drug as a clinical remedy in heart diseases we are struck with this alarming result.

The question that naturally arises then is, what is the cause of this variance in activity and what is the best remedy for it? Is it due to a lack of standardization, or standardization without a uniform method? Is it due to a poor drug supply, carelessness in storing or adulteration, or is it due to the rapid deterioration of the finished preparation? A great deal of work has been done along this line, but I believe we will agree, that while the percentage of the active principles may vary in the leaf and be affected by temperature and light, that if a uniform method was used for the standardization of the finished preparation and this preparation protected from light and temperature it would not show a variance in several years. Roth [4] in a series of tests found that fat-free digitalis deteriorated when kept under ordinary conditions in from five to seven months, and Branson and Sharp,[7] that the alcoholic content would influence its keeping qualities. With these facts in view, then, I believe the solution of the problem for a trustworthy preparation of digitalis would be the provision of a uniform method of standardization and a time limit on all official preparations.

II. BIOLOGIC METHODS.

This leads up to the consideration of the methods employed to-day for the standardization of this valuable drug. I believe we will all agree that digitalis cannot be standardized by chemical means and that a biologic test for its activity is the only way to determine its value. It is then only a question of the best method, and the compulsory adoption of that method to the exclusion of all others. The various methods used for the standardization of the heart tonics are too well

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known to enumerate. I shall only mention those generally employed, and criticise, from a pharmacological standpoint, what I consider to be their weak points.

As we all know, there are several frog methods, all of them based principally on the time it takes to produce standstill of the heart when injected into a lymph-sac. The principal differences in these methods are the time limits and the solution injected. Reed and Vanderkleed's guinea-pig and Hatcher and Brody's cat method both depend upon the toxicity of the drug, or the amount that will prove fatal when injected either subcutaneously or intravenously. Recently we have had suggested another toxic method by Pittenger and Vanderkleed [1] in which goldfish are used. The value of the preparation is determined by the time it takes to produce death of the fish when a preparation of the drug is added to water.

III. CRITICISMS.

After a close analysis of these various methods one is struck first of all with the fact that the potency of the drug is determined by its power to kill or to produce standstill of the frog's heart without taking into account the therapeutic value at all; in the second place, that a drug so poorly absorbed should be injected subcutaneously; and in the third place, the lack of uniformity in the solvents used for injection. Criticisms of the frog methods are that the various species react differently to the drug. The U. S. P. IX recommends the "one hour frog method" for the the Digitalis Group. The animals are subject to climatic conditions and even in the laboratory must be kept at an even temperature during the process of assay. The drug being injected into the lymph-sac may be absorbed with varying rapidity. Roth [4] in a series of experiments reports that temperature effects a marked influence on the toxicity of certain digitalis principles in the frog. (If this be true then a time limit should not be considered an accurate measure of the activity of the drug.) The solvent should play an important part. It may be well to recall that the activity of this drug is due chiefly to three glucosidal principles, digitoxin, digitalin and digitalein, the first mentioned being almost insoluble in water but freely soluble in alcohol. Digitalin is only slightly soluble in water and soluble in alcohol. Digitalein is freely soluble in water. Digitalis also contains another substance, a saponin body, soluble in water but not in alcohol. We are told that this principle makes the other principles, for which the drug is used, soluble in water.

In one of the methods a ten percent infusion of the drug is employed. This preparation should contain all of the principles, but would certainly contain more of the digitonin and less, if any, of the digitoxin. The tincture, preparatory to assay, is usually evaporated on a water bath until at least half of the alcohol is dispelled. Unless this evaporation is carefully carried on the principles, being glucosides, may be partly if not all destroyed.

In spite of the fact then that there is a great difference in the solubility of these principles, we find in the directions for the solutions to be injected, that if a tincture or a fluidextract is to be tested "the greater part of the alcohol" should be evaporated without stating the actual percent. I have never seen published a chemical assay of the amount of digitoxin found in such a solution, but it stands to reason that both digitoxin and digitalin will be thrown out of solution if "the greater part of the alcohol" is evaporated. In the "one hour method" it is recommended that the animal be first tested for susceptibility with a solution of ouabain; although this may be done, still there is an uncertainty as to whether the solution of the digitalis bodies when injected into the lymph-sac will be absorbed with the same degree of rapidity.

My criticisms of the methods in which warm-blooded animals are used are based upon the fact that the amount of alcohol in the solution is not taken into account and that the solution is injected subcutaneously. Haskell [2] found in a series of experiments that alcohol reduced the toxicity of this drug for guinea-pigs and rabbits. The amount of alcohol would influence the quantity of digitoxin and digitalin. Digitoxin causes a vasoconstrictive action and lessens absorption at the place of injection. In this way we would have prolongation of the toxic effect. This method is also an expensive one if guinea-pigs are used, and if the cat method is employed a knowledge of the technic of pharmacodynamics is absolutely necessary.

A criticism of the fish method is that the amount of digitonin in the preparation would influence very materially the toxic effect, it being a well known fact that all saponin bodies have a very toxic effect upon fish. Sollmann [3] says the powerful toxic action of both the sapotoxins and the saponins on fish is due to the rapid absorption of these principles through the gills. Its toxicity then would not show a true digitalis action, but the amount of digitonin present.

I might add that from my observations of the last two or three years in the study of this particular drug, I am convinced that the preparations on the market would not show the variations, as reported, if first of all a uniform method was adopted, the same solvent used, and this method took into consideration the therapeutic effect as well as the toxic value, the standardized preparation being put up in ampoules of doses or in 15 and 30 mil quantities.

It is very evident from the criticisms just enumerated that a slight retardation in absorption, a difference in the amount of the solvent, a difference in the species of animals, or a slight variation in temperature would make a great difference in the time it takes to produce death in the animal.

Against these criticisms of the toxic methods, the argument is advanced that this method is intended as a means of obtaining uniform preparations and not necessarily as a means of proving the therapeutic value. I believe every biologic assay should take into consideration the therapeutic action of the drug, and especially of a drug that is known to contain more than one principle, and when these principles differ in solubility as well as in physiological activity.

IV. SUGGESTED METHOD.

It was with these facts in view that the author three years ago undertook the study biologically of digitalis and its preparations. This investigation led finally to the use of a species of fresh water terrapin or turtle. While there are a number of varieties of this family of *Chelonea,* I have used at least three of the most common ones and find the species whose plastron has two lids, known as Blanding's turtle, the *Emys blandingii,* the most suitable for this work.

My object in using this animal is because of its longevity, its immunity to climatic conditions, the ease of procuring it, and the cost which in itself is quite an item, a six-inch animal weighing about 500 Gm. being purchasable from dealers at $1 per dozen.

I have experimented with the heart of this animal as a means for the standardization of digitalis in every method known to pharmacodynamics, including the perfusion of the isolated heart, the suspension of the heart in an oxygenated solution of the drug, a suspension of ventricular strips in an oxygenated solution of the drug, and the suspension method, the drug being applied to the heart in measured quantities per Gm. body weight every three minutes, these methods having

to do principally with its action on the heart muscle. After a series of experiments I have found that by the injection of the drug into the left aorta (as shown by the drawing of the turtle's heart), an estimate can be made of its therapeutic as well as its toxic value.

As shown by the record in Fig. 1, made with this method, there is, with active preparations, the digitalis action in which the heart is slowed and with this slowing the chambers are more completely filled and at the same time, by its action on the heart muscle, the contractions being more forcible, more blood is thrown out into the circulation. This is known as the therapeutic stage and is the one that the physician desires.

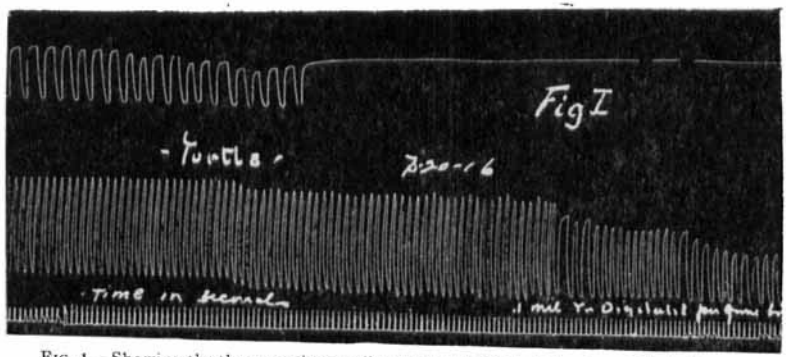

FIG. 1.—Showing the therapeutic as well as the toxic effect of tincture of digitalis, 0.1 mil per Gm. body weight, when injected into the circulation.

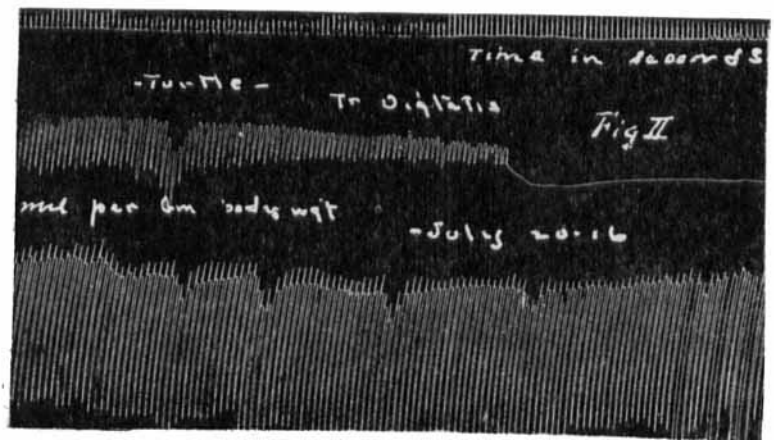

FIG. 2.—Showing the effect of a preparation of tincture of digitalis that is toxic but does not show therapeutic effect, 0.1 mil per Gm. body weight, injected into the circulation.

The method suggested is as follows:

Apparatus—pipette, graduated in hundredths, all-glass syringe or a Hitchen's syringe, kymograph, heart lever, bulldog clamp.

Animal—Terrapin of five hundred Gm. average weight.

Preparation of Solution—Twenty-five percent alcohol, 0.7 percent sodium chloride solution, a sufficient quantity.

Standardization—The turtle is weighed and pithed. The plastron is removed by dissection and the animal tied down. The drug is injected into the right aorta. I have used as a standard the injection of one-tenth of a mil of the solution per Gm.

body weight; this should show the therapeutic effect within three minutes, the heart ceasing to beat, when at least 3 turtles are used, on an average in ten minutes.

Records are made by attaching a string to the apex of the heart. (On account of the peculiar anatomy of the terrapin's heart which is attached at the apex to the pericardium this is easily accomplished). The other end of the string is attached to the heart-lever to record the action on a revolving kymograph. A normal tracing is made. The drug is then injected into the left aorta with a small all-glass hypodermic or a Hitchen's syringe. After the removal of the needle the oozing of the blood is stopped with a bulldog forceps. At the end of from one to

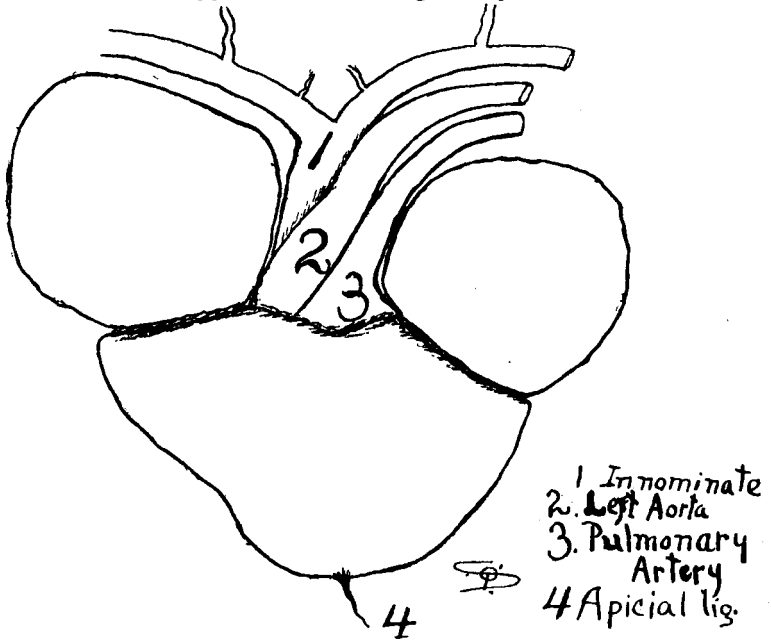

FIG. 3.—Drawing of turtle's heart.

three minutes, if the drug is active, a typical digitalis action will be recorded, the heart ceasing to contract on an average in ten minutes.

Actual standardization then by this method may consist of both a therapeutic as well as a toxic action. After a number of experiments I have found a 3-minute limit for the therapeutic effect and a 10-minute limit for the toxic effect safe to accept. So that if the preparation being tested takes six minutes to show its therapeutic effect and the standard three minutes, then it is only of 50 percent strength. The preparations *must* show a therapeutic effect. I may add in conclusion that I have tested with this method preparations made with different solvents, and find that the best preparation is one made with about 75 percent alcohol, 50 percent of this being evaporated very carefully and made up with sufficient 0.7 percent sodium chloride solution. Lastly, I may add that while I expect to further investigate and perfect this method, the basis on which I claim that this method deserves consideration is given in the following:

V. SUMMARY.

1. The animal is not subject to climatic conditions.
2. Easily procured and kept.
3. The method is simple.
4. Dose depends upon body weight.

5. A record showing the therapeutic effect may be given with the preparation.
6. It shows whether the drug is active therapeutically or not.
7. If a drug is used for its action on the heart muscle it should be injected into or applied to the heart muscle.

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DISCUSSION.

C. E. VANDERKLEED: I think Dr. Zeigler is to be congratulated for his contribution to this very interesting subject. Without any doubt the desirability of determining the therapeutic action of a standard is a reasonable one. It is interesting to point out that nearly all chemical drug assays which are accepted to-day are subject to the same criticism made by Dr. Zeigler. When we determine the percentage of morphine in opium, we by no means determine the therapeutic effect of the opium.

PAUL S. PITTENGER: I would like to ask Dr. Zeigler whether or not any work has been done to prove that turtles do not vary according to season or whether this was just an ordinary laboratory observation?

Before any definite conclusions can be made on this point it is necessary to carry out a series of experiments similar to those we made in determining the variation in susceptibility of guinea-pigs and frogs, namely, to note the variation in the results obtained upon turtles after determining the minimum lethal dose of some standard substance like Ouabain, for example, once a month for one or two years.

I can readily see how the method suggested by Dr. Ziegler might give promising results in determining the qualitative therapeutic effects of digitalis, but before it can be used for quantitative standardization purposes there still remains a lot of work to be done. It will be necessary to prove that the method is sensitive to variations in activity of 8 to 10 percent, as is the case with the methods employed at the present time.

W. H. ZEIGLER: I would like to say in answer to Dr. Pittenger that this method was suggested to me and I am still working on it and have not completed it altogether. I have been working on turtles, and some of the tests, I think, were made at the University about two summers ago. They did not take into account, though, the injection of the drug into the circulation. It had to do with the application of the drug to the heart every three minutes—a certain amount of the drug applied every three minutes.

I have kept turtles without feeding them a month at a time, and I have worked on them in the summer and in the winter. I am sorry I cannot give you the species of turtle on which I worked. I tried to get that before I left, but I did not expect to present this paper here, and I worked on it up until the day before leaving Charleston in order to finish it up. I tried to get this information at our museum, but the officials had all gone away. The particular point I wish to make is that so important a drug as digitalis should be tested and its principal physiological action shown. If digitalis deteriorates, I think it is high time that we put a limit on the preparation. The physician depends a good deal on this preparation. I have had a doctor tell me that he got no action at all, but that may have been due to a lack of observation and so on. We are still in the dark as far as the actual standardization of methods is concerned that would show whether the drug is therapeutically and physiologically active or not.