PHYSIOLOGICAL ASSAY OF GLUCOSIDES, TOXINS AND POISONS ON GOLD FISH, *CARASSIUS AURATUS*

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The object of standardization of the potency of many complex glucosides for which no satisfactory quantitative chemical tests are possible, and the physiological standardization of organic compounds of known constitution, the employment of which depends upon definite potency, the physiological assay of poisons and other related compounds, are among the principal aims of pharmacology. One of the most engaging subjects for the physiologist is the pharmacological study of these agents on the various organs of the body, and the physiological standardization is in many instances a prerequisite to the pharmacological study, to insure working with a compound of definite potency.

It is obvious, therefore, that any method which can insure a greater uniformity and a simplicity of application would be most welcome.

Let us briefly consider for a moment the methods proposed and in vogue for the standardization of digitalis preparations. The U. S. Pharmacopeia recognizes a short method, making use of the frog, *Rana Pipiens*, weighing between 20 and 30 grams and kept at 20 deg., fixing a standard for tincture of digitalis at 6½ milligrams per gram of animal weight as the lethal dose which must cause systolic arrest of the heart. This action must take place by the end of one hour.

Numerous modifications exist of this frog-heart method—the well known Houghton 12-hour method which is of still greater accuracy than the pharmacopeia test. The Focke method is hardly recommendable since it depends upon variable doses to be given throughout the course of the test and, therefore, opens the method to grave chances of inaccuracy. Reed and Vanderlick's Guinea Pig method as modified by Githens and Pittinger, is undoubtedly the finest method of physiological assay for digitalis and digitalis bodies, but it is expensive and time-consuming.

The action of Brody-Hatcher's method is open to the same objection as the method of Focke, in addition to being variable and inaccurate. Pittinger conducted some tests of digitalis on gold fish. The test is made by keeping a gold fish, weighing a gram or over, in 500 c.c. of water at 22 deg. Cent. The minimum lethal dose is that amount of the drug which will cause death by the end of three hours.

This method furnishes the incentive to study the assay on gold fish, and the study has given some very interesting results. By comparison with the guinea pig method we have found that a gold fish

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weighing a gram to a gram and a half in 500 c.c. of water, shows functions exactly the same as an animal of 500 gram weight.

This is the fundamental discovery of our studies. We have found that the 500 c.c. of water in which is placed a gold fish of a gram to a gram and a half functions as an animal weighing 500 grams and with fewer factors of error.

The uniformity of reaction has always been very impressive. Digitalis glucosides or a dose of strychnine, digitalin or any other poison which would cause death within a certain time, would take exactly one-half the time if the amount of water would be decreased to 250 c.c. and if the amount of water were decreased to 400 c.c. the death took place in one-fifth less time, etc.

On increasing the amount of water the lethal qualities were still present but the length of time increased accordingly until the dose was no longer lethal for the larger animal represented by the larger body of water. The test is, therefore, an extremely reliable one for testing the lethal dose per gram of weight of many of the complex glucosides, alkaloids, toxins, poisons, organic compounds, than other methods in vogue. With this method it is easy to maintain the proper temperature, it is easy to observe the effect on the fish at all stages of the drug or poison action, and with carefully developed observation, one can note many interesting actions, some of which will be hereafter related.

Action of Digitalis Glucosides on Gold Fish. The U. S. P. sets a standard of 6½ milligrams on the tincture per gram of frog weight as a standard which should cause arrest of the heart in one hour. When a corresponding amount, 6½ milligrams multiplied by 500 c.c. of water with a gold fish weighing a gram, 3½ grams U. S. P. standardized tincture causes death within 3 hours.

During that time several interesting observations can be made. The digitalis action becomes pronounced, at the end of 2 hours, the fish is no longer able to swim straight but leans to one side and as the digitalis action increases to the point of approaching death he begins to lean on the side more and more until death takes place and the fish completely turns over on the side

From studying different standard tinctures it was found that some, in the first hour, produced a very extensive catharsis, but that some others did not show this action of digitalis on smooth muscle fibres.

These results are very interesting inasmuch as we tried to ascertain to what fraction of the digitalis glucosides it was principally due. Digitalin was tested and while its action was very prompt in producing the stunning of the fish and the swimming on the side and death within 3 hours, or less if the dose was greater than 5 mgs., no catharsis was noted.

Digitalin of commerce, which is a mixture of digitalis principles, mostly digitoxin, also showed very little, if any, catharsis, and while highly potent in producing death within a certain time, according to the size of the dose, the tonic action on the smooth muscle fibres was not produced. We then separated the gitalin faction from the digitalis

and found that this glucoside possesses the tonic action on all smooth muscles, evidenced in the gold fish by extensive catharsis, and that very large doses of gitalin are required to produce death. It was necessary to use three times the tincture dose to produce death with gitalin.

Very interesting is the action of strychnine on gold fish. Two milligrams of strychnine in 500 c.c. of water will produce death of the gold fish in about 2 hours, with extreme opisthotonus. At death the fish is twisted to almost a full curve. Before death supervenes tonic and clonic contractions of the entire body are noticeable. The convulsive movements increase until death supervenes.

The Gold Fish in Immunity Studies. The use of gold fish for studies of immunity and of the potency of toxins and antitoxins has been a source of great satisfactcion and interest.

We have found that in treating a gold fish of a gram and a half in 500 c.c. of water with a diphtheria toxin tested in guinea pigs to a definite lethal dose for a 250-gram guinea pig, the lethal dose per gram of animal weight is the same per gram weight of gold fish and water. This method, in addition to giving more readily controlled tests, eliminating the great variation to which guinea pigs are subject—the possibility of disease, dietary disturbances, etc.—appears to be very superior, and at least an excellent corroborative and collateral method for ready use in research laboratories.

Having established the points above mentioned, we attempted to see if it were possible to induce antitoxic immunity in the fish. Accordingly, fish have been treated with sub-lethal doses of diphtheria toxin for 24 days. At the end of 14 days, and after a period of rest of 2 or 3 days, doses 8 to 10 times the lethal dose have been administered without killing the fish, thereby demonstrating the development of antitoxin production in the gold fish. The availability of the gold fish as a laboratory reagent in the study of toxins and antitoxins opens up great opportunities in the purification studies of antibodies, making it possible for the worker to obtain fairly close results first and then submitting his final product to animal tests.

In the study for the purification of diphtheria and tetanus antitoxins where the bio-chemist would make from 200 to 300 derivatives with the protein structure thoroughly changed, the gold fish method would offer to him a very splendid method of estimating the amount of antitoxin loss in the various chemical manipulations when he had arrived at a satisfactory product, then resort to guinea pig methods for potency determination. Thereby he would have a double check on the potency titration of the purified antitoxin.

Summary and Conclusion. The assay on gold fish, of glucosides, alkaloids, poisons and antitoxins affords an excellent laboratory method of extreme accuracy and which is more easily controlled and more economical than any other method of physiological assay in vogue.

In the studies of the various digitalis glucosides, it can give a better method of establishing lethal dose per gram of weight than any method

known, in addition to furnishing evidence of physiological difference between various glucosides.

The method of physiological standardization of pure digitalis glucosides on gold fish is not advocated as an absolute substitute for the frog heart method and the guinea pig method of assay but as a double check of extreme accuracy which will serve to pick out any pitfalls in the other tests. The gold fish tests for potency of toxins and the antitoxins opens up new avenues of research to the biological chemist in the work of purification of antibodies and elimination of anaphylactogenic and serum sickness producing proteins.