

DISCUSSION.

DR. EDWARD KREMERS:—Is the application of this method new in this country?

DR. PITTENGER:—New, so far as I know.

DR. KREMERS:—Gold fish have been used in other work?

DR. PITTENGER:—Yes, I think they have been used in other work, but so far as I am acquainted with the literature this is the first attempt that has been made to use these animals as a means of quantitatively testing the activity and thereby standardizing galenical preparation or antitoxins.

DR. KREMERS:—I think Dr. Stockberger could tell us something about that. Is he here in the room? Dr. R. H. True worked with gold fish along similar lines some years ago before he went to work in the Department of Agriculture.

DR. STOCKBERGER:—Yes, both Dr. Kahlenberg and Dr. True have conducted work with fish and tadpoles along somewhat similar lines, but the application, of course, was in an entirely different direction. The fish were used merely as a reagent, you might say, in testing the toxicity of various chemical solutions. I do not know that this method has been used in testing galenicals prior to Dr. Pittenger's work.

DR. KREMERS:—The principle was different.

DR. STOCKBERGER:—The principle was different, but the use practically was very similar.

MR. A. J. MEIER:—I would like to ask whether any experiments were carried out to determine whether or not the fish would live from twenty-two to twenty-four hours if left in ordinary water without changing at room temperature when such small amounts of water were employed?

DR. PITTENGER:—No. Such experiments were not necessary as Experiment No. 2 shows that one fish was still alive after being kept for thirty hours in only 300 cc. of a 1-180 dilution of tincture of Digitalis. Table No. 1 also shows that some fish lived for twenty-two to twenty-four hours in this amount of solution.

MR. MEIER:—Without any change of water, or getting oxygen in the water?

DR. PITTENGER:—Yes. It would not be advisable, however, to employ so small a quantity of solution if twenty-four hours should eventually prove to be the best time limit. As stated in the paper the best time limit has not yet been determined. The 300 cc. of solution were merely arbitrarily adopted at the beginning of the experiment when I had no idea as to the length of time which would be required for the fish to die when placed in the different strength solutions employed. It merely happened that some of these fish lived for twenty-four hours. If this should eventually be chosen as the time limit the quantity of the dilution would necessarily have to be increased. The fact that one fish recovered after twenty-four hours shows that such factors had no apparent effect in this time and that by using a shorter end point their effect would be practically nil.

DR. KREMERS:—Wasn't the fish which recovered seemingly dead? Is it hard to tell just when a fish dies?

DR. PITTENGER:—No it wasn't. Apparently it was just as much alive as when placed in the solution. It is very easy to note when a fish dies. It generally first turns over on its side and a little later becomes motionless.

DR. KREMERS:—Did you try to see if you could revive those which were seemingly dead by putting them in salt water?

DR. PITTENGER:—No, I did not as that would have interfered with our results. I know however, that gold fish breeders revive their sick fish by that method.

EXAMINATION OF CALYCANTHUS FLORIDUS FOR ALKALOIDS.

E. R. MILLER AND H. W. BROOKS.

One of the widely-known and popular plants indigenous to the Southeastern States, is *Calycanthus floridus*, a shrub from 2 to 8 feet high, growing from Virginia to Florida and Alabama.

Its chief characteristic, which has won for it almost first place among the wild flowers, is not the size and beauty of its blossoms, but rather their delightful fragrance. To this it owes the almost universal name of "Sweet Shrub."

As a medicinal plant it is not of much importance, but is said to be antiperiodic, stimulant and tonic. The bark has an agreeable, spicy, camphoraceous odor which no doubt suggested the names Carolina allspice, Florida allspice, etc.

Inasmuch as alkaloids have been discovered in the seeds of another species of

the genus, namely, *Calycanthus glaucus*,* we decided to make an examination of the flowers, leaves and roots of the species *C. floridus*.

The roots were dried before extracting, but the flowers and leaves were used in the fresh condition.

About 200 gm. of each of the parts named were extracted by maceration with 95% alcohol which had been distilled over tartaric acid. The filtered alcoholic extract was distilled on a water bath until the greater part of the alcohol had been removed. The residue was then treated with water until no further precipitation resulted. The resinous and oily matter was removed by filtration and the filtrate evaporated to a small volume on a water bath. In the case of the root this aqueous extract was rather milky in appearance, and was cleared up by shaking out with ether. The aqueous liquid was then made slightly alkaline with ammonia and extracted in succession with ether, chloroform, petroleum ether and benzene. After evaporation of the immiscible solvent the residue was treated with a small amount of water acidulated with dilute H_2SO_4 , and tested with general alkaloidal reagents. The following results were obtained:—

SOLUTION FROM ETHER EXTRACT.

	Roots	Flowers	Leaves
Potass mercuric iodide	Slight ppt.	Yellowish White ppt.	Heavy White ppt.
Co. Solution iodine. . .	Reddish ppt.	Heavy Brown ppt.	Red ppt.
Potass Bismuth iod. . .	Red ppt.		
Tannic acid.	No ppt.	No ppt.	No ppt.
Phosphomolybdic acid	No ppt.	No ppt.	Heavy White ppt.
Picric Acid.	No ppt.	Yellow ppt.	Yellow ppt.

The chloroformic extract of the flowers gave precipitates with phosphomolybdic acid and solution iodine compound. The petroleum ether extract of the flowers gave a ppt. with sol. iod. comp. The benzene extract of the leaves gave a white ppt. with potass. mercuric iod.; a red ppt. with sol. iod. co. and a greenish-yellow with picric acid, and the ether extract from the leaves gave a heavy white ppt. with potass. mercuric iod.; a red ppt. with co. sol. of iodine; a heavy white ppt. with phosphomolybdic acid and a yellow ppt. with picric acid.

The results obtained make it highly probable that the plant contains alkaloids, especially in the leaves, the extract from which produced the most abundant precipitation with the alkaloidal reagents. Considering the nature of the solvents used in preparing the solutions to be tested it is scarcely probable that enough protein matter could have been present to give the abundant precipitation that was obtained in several cases.

The subject will be further investigated as soon as sufficient material can be obtained.

Alabama Polytechnic Institute.

* Dr. R. G. Eccles, Proc. Am. Pharm. Assoc., 1888, 84, 382.

Dr. H. M. Gordin, Proc. Am. Pharm. Assoc., 1904, 345; 1905, 224; 1908, 805; 1909, 889.
Jour. Am. Chem. Soc., 1905, 144.