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Ichthyometric Studies on Some Mercurials

*By David I. Macht and Elizabeth C. Spencer**

INTRODUCTION

Fish have been employed as test objects for pharmacological work by various investigators. In fact, they are much more useful for both bioassay and for research purposes than most pharmacologists appreciate. Larger fish have been employed for the study of drugs on the circulation in the gills and the effect of various chemicals on the heart. It is the small fish, however, which are especially useful for testing the toxic effect of drugs on different physiological functions. Thus, small fish have been used advantageously for the study of local anesthetics (1), for the assay of picrotoxin (2), for testing the potency of digitalis (3) and for the detection of estrogenic (4) and androgenic hormones (5). Recently, goldfish have been recommended for the bioassay of thyrotropic hormone (6) and also for the study of the biophysical effects of x-rays (7). Macht with Craig used goldfish in a comparative study of nicotine derivatives (8) and with Leach employed them also for quantitative comparison of a long series of isomeric octyl alcohols (9). In connection with the last-named research, a graphic method for recording the activity of goldfish in solutions of various drugs was described. The present writers have employed the same ichthyometric technique in studying the effects of opium alkaloids, snake venoms and numerous other drugs. The results of this investigation will be published in due time. In the following paper the authors describe their study by this quantitative ichthyometric method of the toxicity of a series of mercury compounds, the results of which are of both scientific and practical interest.

EXPERIMENTAL

Technique.—The writers studied the behavior of goldfish (*Carassius auratus*) with an ichthyometer registering their movements on a slowly revolving kymograph. Figure 1 is an outline of the simple apparatus, a modification of the ichthyometer originally described by Spencer in 1929 (10). The set-up consists of a round granite or enamel bowl filled with water to a depth of one and a half to two inches. Two crossed metal springs are so placed over the top that the hole made at their point of juncture is directly over the center of the bowl. Goldfish approximately two inches in length from snout to root of tail are used for these tests. A silk thread is drawn through the muscle just in front of the dorsal

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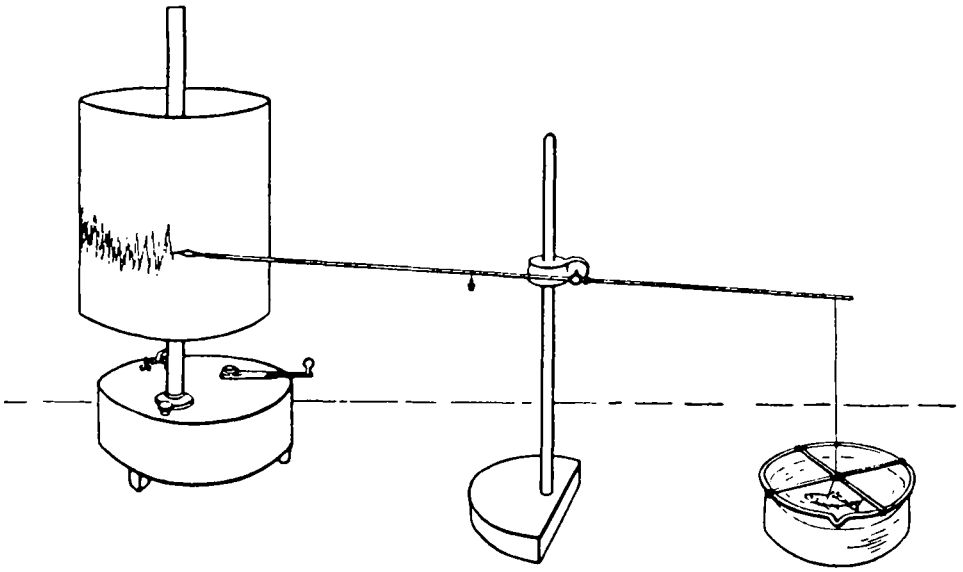

Fig. 1.—Ichthyometer.

fin and tied in a short loop, the other end being passed through the opening made where the bars cross and secured to one arm of a long lever. The other arm is lightly weighted to balance the two arms on the pivot and the fish's movements result in the weaving of a pattern on the slowly revolving kymograph. The length of the lever arm is adjusted, so that the stylus records on the drum not only the frequency but also the amplitude of lever contractions. The shallow water in the round bowl renders the vertical or diving movements of the fish negligible while its motions in horizontal directions are fully recorded.

This apparatus can be employed for study of the behavior of fish under the influence of any kind of drug or chemical, whether sedative or convulsant. When necessary, the fish can be screened from light by blotters placed over the top of the bowl. When studying the normal movements of the suspended goldfish as well as their behavior while under the influence of various drugs, the authors place in the bowl a second fish as companion to that recording its contractions. Quiescent periods may thus be effectually eliminated and the effect of the drug used can be studied on the unencumbered goldfish as well as on that harnessed to the lever.

Effect of Various Mercurials.—A number of mercury compounds—*i. e.*, mercuric chloride, mercuric cyanide, mercurochrome or dibrom-oxy-mercuri fluorescein, merodicein or mono-hydroxy-mercuri-diiodo resorcinsulphonphthalein, and flumerin or oxy-mercuri fluorescein—were thus studied on goldfish; and the results obtained were extremely interesting. It was found that the organic and inorganic mercury derivatives varied greatly in their toxicity for fish. The three organic compounds, mercurochrome, merodicein and flumerin, all proved to be relatively little toxic for goldfish. Thus, for instance, 1:10,000 solutions of mercurochrome were usually innocuous and goldfish placed therein survived for 24 hours. Even when placed in solutions as strong as 1:5000 of mercurochrome, the goldfish survived from 12 to 15 hours. Identical results were obtained with solutions of flumerin and merodicein. Very different, on the contrary, were the effects of inorganic mercurials, very dilute solutions of which soon proved toxic for goldfish. Healthy specimens, placed in 1:100,000 solutions of mercuric chloride, died in a few hours. Even 1:200,000 dilutions of bichloride were fatal for these fish. Solutions of mercuric cyanide, of course, were more toxic for goldfish.

Of greater interest were the results obtained with combinations or mixtures of an organic with an an inorganic mercurial. In special studies made with a combination of mercurochrome and bichloride of mercury minute traces of the latter potentiated the toxicity of the mercurochrome solution, *i. e.*, effected a synergism, indicated by heightened toxicity of the *mixture* as compared with that of the individual constituents. The activity of goldfish placed in such solutions was speedily inhibited and death followed. Such a difference in behavior of goldfish in solutions of pure mercurochrome and of mercurochrome containing traces of inorganic mercury salts, respectively, obviously suggests a delicate biological method for discovering impurities in oxy-mercuri-dibrom fluorescein and also the spurious preparations of mercurochrome occasionally foisted on the market.

The subjoined ichthyograms or tracings made by goldfish placed in the various solutions described above strikingly illustrate the results obtained. In each case the upper curve was traced on a kymo-

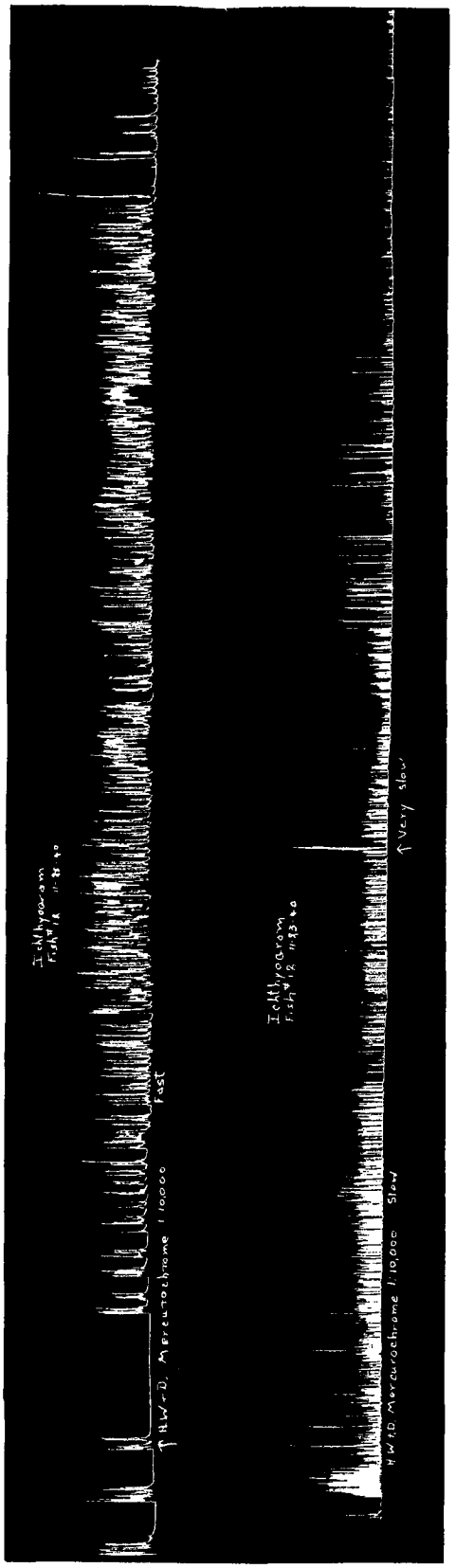

Fig. 2.—Ichthyogram of Effect of 1:10,000 Mercurochrome Solution.

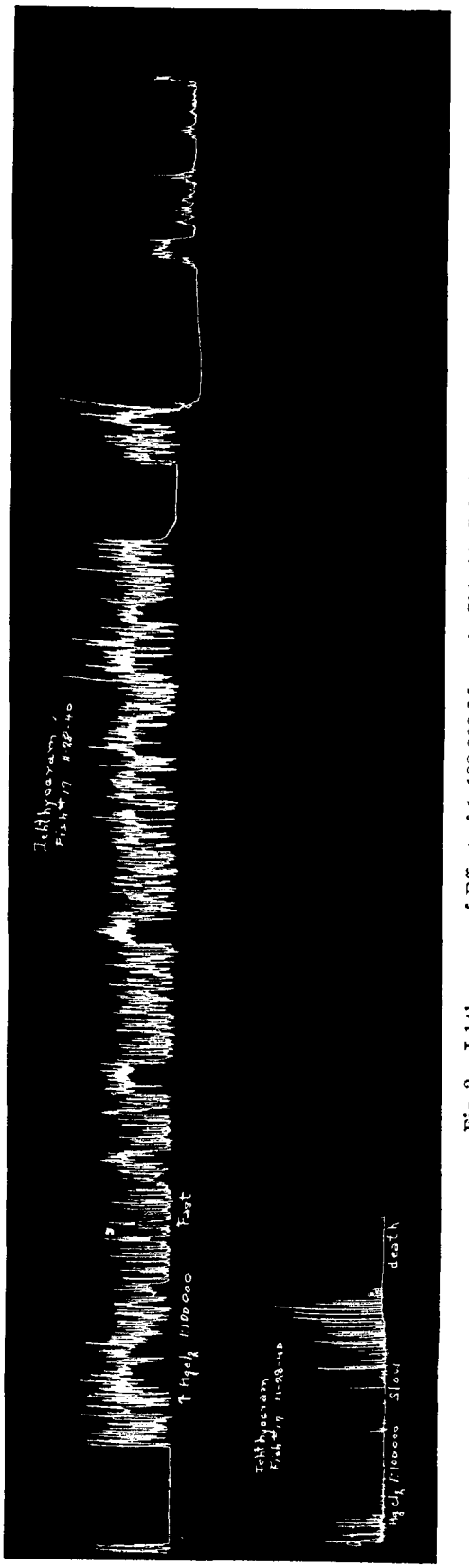

Fig. 3.—Ichthyogram of Effect of 1:100,000 Mercuric Chloride Solution.

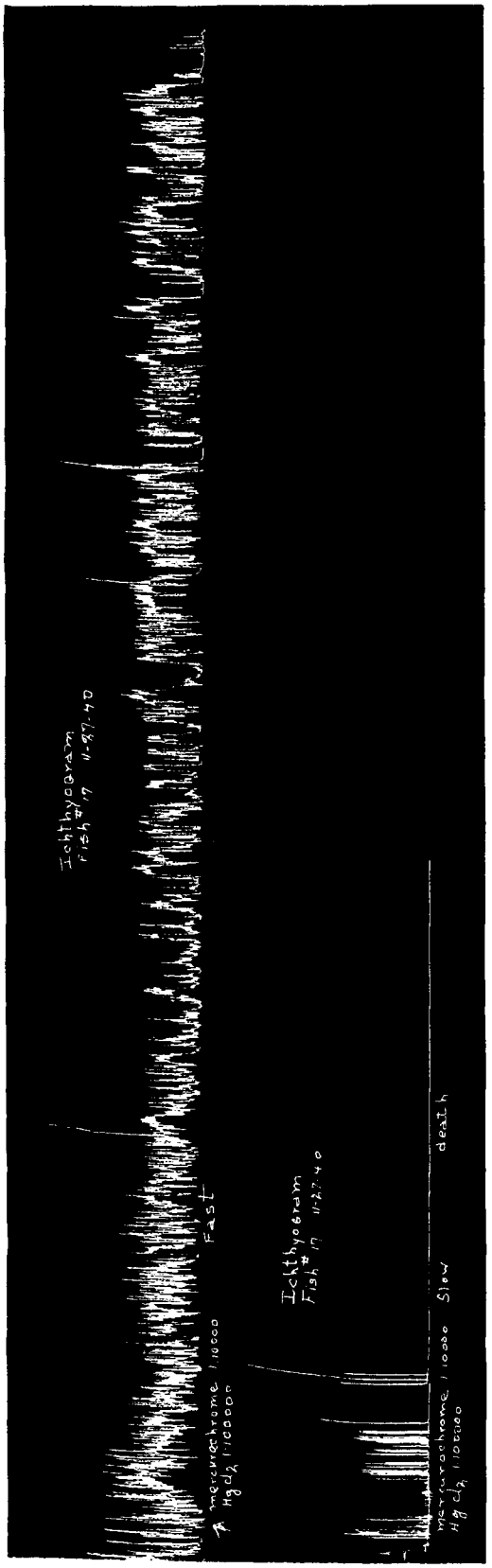

Fig. 4.—Ichthyogram of Effect of a Mixture of 1:10,000 Mercurochrome Solution and 1:100,000 Mercuric Chloride Solution.

graph making one revolution an hour whereas the lower was made on a much slower drum running overnight. Figure 2 is an ichthyogram produced by a goldfish placed in a 1:10,000 solution of mercurochrome (H., W. & D.) and surviving for 24 hours. Figure 3 shows the effect of a dilute solution of mercuric chloride (1:100,000), which caused death in a few hours. Figure 4 reveals the results obtained with a solution containing a mixture of mercurochrome, 1:10,000, and bichloride of mercury, 1:100,000.

SUMMARY

1. A simple method for graphically recording on the kymograph the neuromuscular activity and general behavior of small fish has been described.

2. This method has been employed by the authors for pharmacological study of the comparative effects of various drugs on goldfish.

3. A striking difference was noted in the ichthyograms produced by goldfish placed, respectively, in solutions of certain organic mercurials and of inorganic mercury salts, the former being much less toxic than the latter.

4. The ichthyometric method has proved useful not only in differentiating between organic and inorganic mercury compounds but also in discovering the presence of inorganic mercury contaminants in mercurochrome.

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"To be guided by reason is to obey the laws of nature."—Ernest Wood

Non-Toxic Character of Ursolic Acid.*

Preliminary Study

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INTRODUCTION AND REVIEW OF LITERATURE

Ursolic acid is a monohydroxytriterpene acid (1) of the formula $C_{30}H_{48}O_3$ (2). It is widely distributed in nature, having been found in *uva ursi* leaves (3), mistletoe (4) and the skins of apples (5), pears (6) and cherries (7). Cranberry pomace obtained in the commercial canning of cranberry sauce was also found to contain ursolic acid (8). According to Winterstein and Stein (9), ursolic acid in the form of a saturated solution of its sodium salt is toxic to fish. This reported toxicity to fish might be due to the use of a saturated solution or possibly to still other factors. As ursolic acid is being introduced as an emulsifying agent in pharmaceutical and food preparations, it was thought advisable to determine whether or not it possessed toxic properties.

EXPERIMENTAL

Description of Sample.—The ursolic acid used in this study was prepared from cranberry skins.[1] This ursolic acid was a fairly pure sample although it had not been crystallized from alcohol. The yield of ursolic acid manufactured from dried cranberry skins is about 10 per cent.

Toxicity.—Rats, guinea pigs, chickens and rabbits were fed ursolic acid orally at levels of from 1000 to 5000 mg. per Kg. of body weight. These animals were placed in individual cages with abundant drinking water, but were left without food for 24 hours. The ursolic acid was then fed mixed with dried bread crumbs in the case of the rats and guinea pigs, laying mash in the case of the chickens and Purina Fox Chow (a prepared dry feed) in the case of the rabbits. The animals were observed for 12 days during which time no toxic manifestations were noted. Autopsies were then performed on a representative number of animals. No abnormalities were evident; therefore, it would seem that ursolic acid is not injurious to these animals when fed orally in large quantity. The liver, kidneys, heart, lungs, adrenals, stomach, intestinal tract and ovaries (in the case of females) were carefully examined

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[1] Ursolic acid obtained through the courtesy of Cranberry Canners, Inc., South Hanson, Mass.