The results appear tabulated as follows:

Process.....	1. Percent.	2. Percent.	3. Percent.	4. Percent.	5 U. S. P. Percent.	6. Percent.	7. Percent.
1st.........	56.9	62.1	64.6	65.2	65.3	64.9	65.8
2nd........	57.2	62.0	64.7	65.0	65.0	64.7	66.1

The tabulation indicates that process VII yields the best results, while the U. S. P. and process IV yield very nearly the theoretical amount of the process alcohol present.

LABORATORIES OF SHARPE & DOHME.

THE REACTIONS OF GOLDFISH TO CERTAIN HABIT-FORMING DRUGS—THE USE OF THE GRADIENT TANK.

BY VICTOR E. SHELFORD.

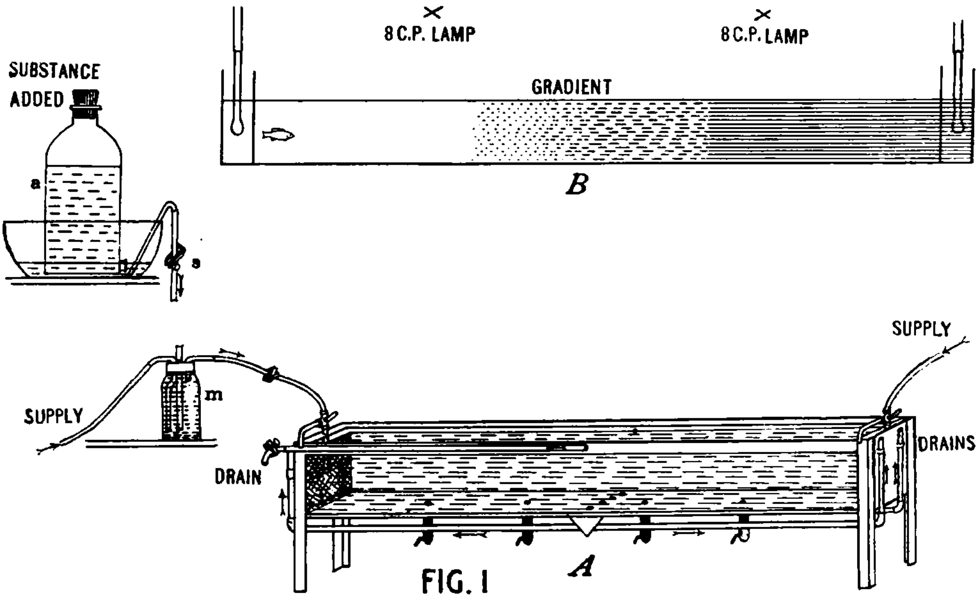
The problem of the cause of the development of the drug habit, *i. e.*, of the peculiar effects of habit-forming drugs which cause the user to crave them, has been approached from several different angles, but none of them have afforded a solution. Attention appears to have been directed especially toward morphine and related compounds.[1] The theory that morphine causes the formation of oxymorphine, which produces an effect the opposite of morphine, has been exploded by the discovery that oxymorphine is not formed and that its effects are not the opposite of morphine. The idea that immunity is developed is rejected because of the fact that such immunity does not exist in animals which are habituated by the use of morphine. The increased capacity of the organism to destroy morphine is not an adequate explanation as morphine is very slowly destroyed.

Most of the work has been done on mammals, some on frogs, but I find almost nothing in the literature that shows the development of anything like a craving, taste, or preference for the substance in question, on the part of the lower vertebrates. In fact it is not easy to show that these animals are so affected. By chance the writer discovered that fishes are peculiarly affected by numerous organic substances in aqueous solution, when put under special experimental conditions. These conditions are established in a long, narrow tank, 122 cm. long, 15 cm. wide and 13 cm. deep (see Fig. 1-A), in which water containing a drug flows into one end and out at both top and bottom, at the middle, while water which contains none of the drug flows into the other end at the same rate. The two flows meet at the middle and with most substances there is a mixture of the two kinds of water which occupies the center third of the tank. In this mixture a fish moving from the pure water end toward the drug-containing end encounters a gradual rise in concentration of the drug. This region of change of concentration is called the gradient. The character of the gradient in these tanks has been fully determined, by taking samples, by measuring conductivity, and by the use of colored water. Usually a sample which contains none of the salt or any amount from the faintest traces up to the full concentration introduced, may be withdrawn (Fig. 1-B). Some substances diffuse almost through-

---

[1] Sollman Manual of Pharmacology, 1917.

out the tank, so that the gradient, or region in which there is a rise in concentration extending from near the pure water end toward the end in which the drug is introduced, reaches almost the entire length of the tank.



GRADIENT TANK (A). LONGITUDINAL SECTION OF TANK (B).

Figure 1-A:—The gradient tank and apparatus for introducing drugs into one end. The water flows into the two ends of the tank from a common source. The flow is adjusted with a pinch cock on a rubber hose at the right-hand end, for example, at 500 Cc. per minute. This is done by turning the 3-way valve so as to run the water outside of the tank through the small spout which ends at the water level just outside of the tank. The water can be caught here in a graduate for a definite length of time and the flow per minute determined. The flow at the end into which the drug is added may be set at, say, 400 Cc. per minute and then sufficient of the drug solution added to the mixing bottle from the siphon above at the left (100 Cc.) to make this 500 Cc. also. The solution of a non-volatile drug is siphoned (see Fig. 1-A) from dish in which is a 12-liter aspirator bottle (*a*) with the upper opening tightly corked and the lower one open. When the water in the dish falls below the level of the lower opening a few bubbles of air slip in and the same amount of fluid flows out, thus maintaining a constant level in the dish as long as the supply in the aspirator bottle holds out. Volatile substances have usually been added directly from the lower opening of the aspirator bottle. In this case it is necessary to correct the flows occasionally. The drug solution is run into a mixing bottle (*m*) which is connected in the flow of pure water.

Figure 1-B shows a longitudinal section of the tank when a substance is introduced at the left-hand end. The substance is shown by black markings. The central portion shows a gradient between pure water (white) and black lines. The graphs are drawn on the basis of the position of the fish in this longitudinal section.

In order to discuss the behavior of fishes in habit-forming substances, it is first necessary to note in detail what happens when (1) a fish encounters no change in water and (2) when it encounters water containing a quantity of carbon dioxide or other environmental substance. When the water is the same throughout fishes usually go back and forth in the tank either without turning around in the middle or, if so, without showing any preference for either end. Graph 1 shows the usual movement of an active goldfish which went back and forth, and though it turned around in the middle third of the tank occasionally, it turned the same number of times when headed each way, even in the short 20-minute period. Graph 9 shows a control graph in which an inactive fish staid near the center. Graph 10 shows the behavior of a minnow which swam from end to end, except for stopping under the lights—a reaction quickly masked by response to another stimulus. When introduced into a tank in which the water running into one end is charged with carbon dioxide up to about 15 Cc. per liter, or more, the fishes act in a very striking and characteristic way (2). When they swim into the acid water, the fishes undoubtedly sense deleterious concentrations of CO_2 upon entering it. They give evidence for this by the following activities, as shown by an earlier set of experiments:[2] A definiter eflex was often given by minnows (*Abramis, Notropis, Hypopsis*) and sunfishes (*Lepomis*), the first time they entered the modified water. The fish suddenly stops, backs quickly a few millimeters, and then starts ahead again, often repeating the reflex before going farther forward. In an earlier paper, this was called the backing-starting reaction. This may be due to stimulation of the nostrils, as Sheldon[3] states that stimulation of the nostrils of the dogfish results in a quick jerk of the head. There is acceleration or increased vigor of movement of fins, tail, or body, which begins at once or after a very short time. Sheldon found that the application of solutions to these parts caused them to be moved. The opercles were lifted, the lower jaw protruded, or the mouth moved in a manner characterized as coughing, gulping or yawning. Sheldon found that stimulation of the mouth or spiracle gives rise to violent gulps. In our experiments these reactions occurred singly or in combination. The time necessary to produce them was variable, but depended upon the strength of the stimulus, which confirms further observations by Sheldon.

When a fish enters a deleterious solution of alcohol, cocaine, morphine, or any one of the several other substances, nothing of the kind ordinarily happens. There is no rejection of the drug containing water, no gulping or raising of the opercles. The fish swims into the water apparently without noting any change. After a few entrances it begins to turn back from the normal, pure water, or from the lower concentrations of the drug in solution just as from carbon dioxide, but usually without the gulpings, or any of the other movements which follow stimulation. This was first noted in the study of the reaction of the golden shiner to ammonia in 1911.[4] It was again observed in the study of the reactions of a number of species of fish to coal-tar products in connection with a study of the effects of stream pollutions.[5] Fishes of various species showed a *preference for*, that is, *reacted positively to*, coal-tar products, various mixtures of them, and gas liquor. The species giving positive reactions were the large-mouthed black bass, minnows (*Notropis, Pimephales, Abramis*), rockbass, and three species of sunfish.

The substances for which the fishes showed the preference as described, and to which they are said to react positively, are the following: Ammonia and

[2] Shelford & Allee, *Jour. Expt. Zoöl.*, 14, 208–256; *Jour. Animal Beh.*, 4, 1–30.

[3] Sheldon, *Jour. Comp. Neurol.*, 19, 273–331.

[4] Shelford and Allee.

[5] Shelford, *Bull. Ill. St. Lab. of N. H.*, 11, 383–411.

several of its salts, aniline, pyridine, isoquinoline, sulphur dioxide, carbon disulphide, thiophene, acetone, phenol, paracresol, phenanthrene, naphthalene, xylene, toluene, acetylene, illuminating gas, and waste.

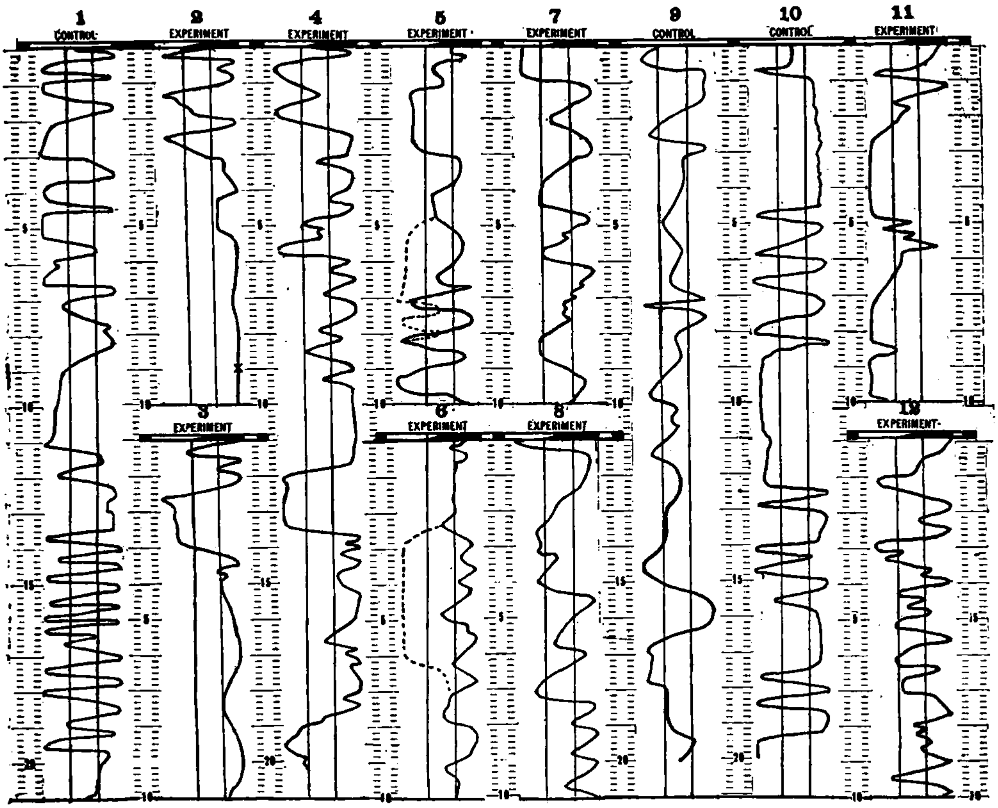


CHART 1.

The graphs on this chart, numbered 1, 9, and 10, show the movements of fishes in the gradient tank when no drug has been added at the end, and the water is therefore of equal purity throughout.

The gradient tank is shown in Figure 1-A. Figure 1-B is a diagram of a longitudinal section of the tank; the left-hand end was used for the introduction of water such as the fishes had been kept in, and the right-hand end was used for the introduction of water to which the substance being tested had been added. The light vertical lines are intended to indicate the location of thirds of the tank length. The solid black area at the right between the two lines at the head of each graph is intended to show the part of the tank in which the drug solution is nearly the full strength of that flowing in, and the narrowing of this black area from right to left in the middle third is intended to indicate the region of principal gradient; X indicates that the fish became intoxicated.

Graph 1 shows the usual back and forth movement of a goldfish when not encountering any difference in the water in the two ends—a control experiment.

Graph 2, positive reaction of a goldfish to water containing 0.35 Gm. cocaine hydrochloride per liter introduced at the right. The fish turned back on encountering a slight dilution after 40 seconds, swam the entire length of the tank twice, turned back on encountering a slight dilution at the end of four and a half minutes and remained in the highest concentration of the drug until intoxication resulted.

Graph 3, the same as the preceding, but a different fish; shows a strong preference for the cocaine solution and an avoidance of the pure water.

Graph 4 shows a positive reaction of a goldfish to a 1.5 percent solution of ethyl alcohol introduced at the right-hand end. The fish turned back repeatedly from the pure water after trying out the tank during the first three minutes. A graph of the reaction to 10 percent alcohol resembled No. 3 very closely.

Graph 5, an indifferent reaction of two goldfish to 0.15 Gm. per 1 Gm. of morphine hydrochloride. Where the two fishes moved together only one line appears; where separately, one of them is indicated by a broken line.

Graph 6, a positive reaction of two goldfish to 1 Gm. per 1 Gm. of morphine hydrochloride per liter. One fish was negative to the water without morphine from the beginning; the other spent three minutes in the pure water beginning at the end of three minutes.

Graph 7 shows a positive reaction of a goldfish to water half saturated with naphthalene; Graph 8, to saturated naphthalene. In both cases the fish became positive to the naphthalene at once.

Graph 9 shows a control movement of a sluggish goldfish.

Graph 10, a control movement of a wild minnow (Pimephales).

Graph 11, a negative reaction of such a minnow to 1–2 percent alcohol.

Graph 12, a positive reaction after the fish had been kept for 30 minutes in a 0.5 percent solution of alcohol.

Fishes are not invariably positive to all of these, as the positive reaction is often determined by concentration, character of the dissolving water, etc., but positive reaction is the usual rule. While ether, chloroform, chloral, and paraldehyde are habit-forming in the case of man, I find no records in the general works for any of the coal-tar products noted above. Since the goldfish has been used as a test animal in drug standardization, it was thought desirable to test its reactions to two or three habit-forming drugs. Cocaine hydrochloride, morphine hydrochloride, and ethyl alcohol were chosen. The standard gradient tank with symmetrical incandescent lights and a hood were used with general results as described on p. 598. Because of the large amount of water used, it has been impracticable to carry on the experiments in distilled water. They were conducted in water such as the fishes had been kept in.[6]

COCAINE.

Only a few experiments were tried with this as the fishes were positive to all concentrations tried. In 0.35 Gm. of the hydrochloride per liter the goldfishes were positive at once, *i. e.*, turned back on encountering the pure water (Graphs 3 and 4). They became intoxicated and died in the high concentration end. No reactions such as follow peripheral stimulation with CO_2 were noted. The graphs show that after two or three minutes' exposure and a few turnings they remained in the higher concentration of the drug.

ETHYL ALCOHOL.

Goldfish were positive to 1.4 percent alcohol, as shown in Graph 4. Waters with less alcohol or no alcohol were quite consistently avoided. Their reaction to 0.5 percent is usually negative. Their reaction to 1 percent alcohol was positive and remained so consistently for two hours, when some nervousness and excitability occurred which lasted for five hours more in which the fishes spent most of their time in the more dilute alcohol of the center of the tank, but avoided

[6] For analysis and comment see Wells, *Biol. Bull.*, 29, 226–227.

the pure water. When 2 percent alcohol was used they remained clearly positive for six hours. With 3 percent alcohol they remained clearly positive for three hours, when they became nervous and slightly tipsy. In this condition they appeared not to note the difference between the two ends, but still spent most of the time in the alcohol end. Their reaction was decidedly positive to 10 percent alcohol. They occasionally moved toward the low concentration but turned back quickly on encountering a slight decrease in concentration. At the end of 20 minutes they were semi-intoxicated and the experiment was discontinued. In 20 percent alcohol they avoided the full strength but still gave a positive reaction for the short time they were under observation.

MORPHINE.

They appeared not to note 0.15 Gm. per liter of morphine hydrochloride, as shown by Graph 5, where the drug was not avoided, but no good evidence of any preference shown. In 1 Gm. per liter one fish showed a positive reaction throughout, while the other spent a little time in the pure water. Other individuals avoided the strongest morphine and pure water staying in the center of the tank and turning back from both ends.

NAPHTHALENE.

Goldfish are positive to half saturated and saturated solutions as shown in Graphs 7 and 8. It is only very slightly soluble but sufficiently so to kill fishes in an hour or more.

OTHER SPECIES.

Minnows of the genus *Pimephales* were found, in course of my experiments on pollution, to be more often negative than other species. This species was uniformly negative in reaction to alcohol up to three percent. After being kept 20 hours in $^1/_4$ percent it was negative to 1 to 2 percent, more negative than when kept in water. Fishes kept one-half hour in $^1/_2$ percent gave positive reaction to 1 to 2 percent; others kept 20 hours in $^1/_2$ percent showed a more positive reaction, showing that the preference for alcohol is developed by exposure to it, but that the fish is negative to concentrations to which the goldfish is positive.

TADPOLES.

A single experiment with a tadpole and 20 percent alcohol showed the animal positive and suggested the use of tadpoles about to transform into frogs as affording a well-known laboratory animal for such studies.

CONCLUSIONS.

These incidental preliminary observations indicate the following tentative general considerations:

a. That fishes quickly form a preference for water containing alcohol, cocaine, morphine, and certain other substances. They appear, as a rule, to avoid very weak concentrations and are in nearly all cases less positive at first. This with the habituation of minnows usually negative to alcohol by an exposure of 30 minutes in $^1/_2$ percent alcohol indicates that the sensory endings are quickly modified in their sensitivity.

b. The gradient tank may afford a means of studying "habit" formation and also of investigating compounds or combinations of drugs which may produce the desired therapeutic effect with least danger of creating an appetite. Results with fishes may differ from those obtained with man, but they may readily afford information which can serve to interpret results with higher forms.

ZOÖLOGICAL LABORATORIES,
UNIVERSITY OF ILLINOIS.

RAPID GENERAL ASSAY METHOD FOR ALKALI SALTS OF ORGANIC ACIDS.

BY A. B. LYONS.

Ignition of salts of organic acids is a tedious operation, even if one is satisfied merely with thorough carbonization of the organic compound. In the official general assay process (U. S. P. IX, p. 589) this is all that is required, yet the plan of dissolving out from the carbonized residue the alkali carbonate with aid of volumetric acid and heat, filtering out the carbon and washing out all the residual acid, calls for a considerable expenditure of time and labor.

It has been suggested by E. Elvove that conversion of the salt into a *sulphate* is to be preferred to simple ignition resulting in the formation of a carbonate, and no doubt equally good results can be obtained by this procedure. There is, however, no gain in time consumed, but by a very simple modification of the process it is made far more rapid than either of the other methods, with no sacrifice of accuracy in the results.

The following is the new procedure: Weigh accurately in a small beaker about 0.5 Gm. of the salt, add 20 mils of alcohol, to which has been previously added 10 drops of strong sulphuric acid, from a pipette delivering 60 drops to the mil. Stir the mixture well and let it stand a few minutes, then decant the alcoholic solution into a platinum or quartz dish. Wash the residue in the beaker by decantation with two successive portions of alcohol (5 and 3 mils), adding the washings to the dish. Set fire to the alcohol and allow it to burn off, then ignite the residue at a temperature not exceeding dull redness. Since the residue consists almost wholly of the organic acid of the salt, together with a little free sulphuric acid, the carbon will burn off, in a very short time, the ignition requiring no attention meanwhile.

Dissolve the residue of alkali sulphate in the beaker in a little hot water, and when the carbon has been practically all burned off from the first residue, cool the dish and add to it the sulphate solution, together with rinsings from the beaker. Evaporate the solution in the dish to complete dryness and ignite at a red heat until white. Cool in a desiccator and weigh as alkali sulphate.

Since the sulphate has not been in contact with carbon to any appreciable extent during the ignition it may be considered to consist (barring impurities in the salt) wholly of alkali sulphate. However, if there is any doubt about this, the salt may be dissolved in a little hot water, a drop or two of sulphuric acid added, the solution evaporated, and the residue once more ignited.