Estimation of Low Chlorpromazine Concentrations
by Surfacing and Sinking Reaction of Minnows
(Gambusia affinis)

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Abstract. Surfacing and sinking reactions of minnows (Gambusia affinis) allow the determination of chlorpromazine in aqueous solutions down to concentrations of 0.1 µg/ml. In a given volume of solution, neither sex nor number of the fish per tube have any effect on their response to the low doses. A twenty-fold augmentation in volume causes a two-fold increase in sensitivity. The time elapsing, from the beginning of immersion until onset of surfacing, increases with decreasing drug concentration. Partial recovery from the drug is obtained after washing of fish with tap water. Adaptation occurs when the fish are immersed for more than 48 hrs.

Key-Words: Estimation — Chlorpromazine — Minnows.

Several psychotropic drugs have been shown to affect the normal behavioral pattern of fish (Abramson and Evans, 1954; Evans et al., 1956; Walaszek and Abood, 1956; Abramson et al., 1961; Cutting et al., 1959; Keller and Umbreit, 1956). The modifications in behavior are produced by injecting or feeding the drug or by placing the fish into its solution. A characteristic behavioral feature of various fish, when under the influence of either LSD-25 (Abramson et al., 1961) or chlorpromazine (Cutting et al., 1959; Saxena et al., 1962), is their tendency to accumulate near the surface of the liquid and to remain there relatively immobile. It was found by us that, in addition, such drugged fish, after surfacing, lose their natural tendency to sink when alarmed by an outside disturbance.

The present paper examines the possibility of utilizing the surfacing-sinking response for the estimation of low concentrations of chlorpromazine (CPZ) in aqueous solutions. The minnow (Gambusia affinis), which proved to be a suitable species, was employed throughout this study.

Animals and Apparatus. The minnows were taken from ponds in the vicinity of our institute. Both sexes were used indiscriminately, and their

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length varied in the range of 10—30 mm. The fish were kept, without aeration, in shallow plastic containers filled with tap water and fed on mosquito larvae.

The apparatus consisted of an illuminated wooden stand holding 21 glass tubes, each of about 120 ml volume (20 cm long, 3 cm in diameter).

Procedure and Results

All experiments were conducted at room temperature (22°C) and lasted for either 24 hrs or 48 hrs.

The fish were transferred from the plastic containers into the glass tubes—each containing 100 ml fresh tap water and 2—5 minnows—and allowed to become accustomed to their new surroundings for about 2 hrs. Thereafter, the water in each tube was replaced by 100 ml of the pertinent test solution (CPZ in tap water, at concentrations ranging from 0 to 6 μg/ml).

In the control tubes, which contained tap water only, the fish were evenly distributed. In CPZ solution, the fish tended to accumulate near the surface. The Table summarizes the results of a typical experiment which shows that the time elapsing, from the beginning of immersion until the onset of surfacing, increases with decreasing drug concentration.

<table>
<thead>
<tr>
<th>Concentration of CPZ (μg/ml)</th>
<th>Time of surfacing (min)</th>
<th>Mortality within 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>5</td>
<td>25/25</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4/25</td>
</tr>
<tr>
<td>1.5</td>
<td>20</td>
<td>0/25</td>
</tr>
<tr>
<td>0.5</td>
<td>30</td>
<td>0/25</td>
</tr>
<tr>
<td>0.2</td>
<td>60</td>
<td>0/25</td>
</tr>
<tr>
<td>0</td>
<td>&gt;3600</td>
<td>0/25</td>
</tr>
</tbody>
</table>

Length of fish: 15 mm. Each tube contained 5 minnows; 5 tubes were employed for each concentration.

A concentration of 6 μg/ml was lethal to all the fish within 65 min following exposure. At 3 μg/ml, a mortality of 16% occurred within 24 hrs. At lower concentrations, no mortality was observed within the latter period. Minnows longer than 30 mm could not be used with the 100 ml volume test tubes for periods exceeding 48 hrs since by that time they tended to surface in tap water, probably due to lack of oxygen.

The symptoms of surfacing remained evident for at least 48 hrs; after this time, the effect started to wear off, presumably owing to