STIMULATION BY HYDROCHLORIC ACID AND BY THE
NORMAL ALIPHATIC ACIDS IN THE SUNFISH
EUPOMOTIS

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A study of chemical stimulation in aquatic animals involves a
correlation between the chemical nature of the compound under
consideration and the changes its presence may produce upon the
responsive organism and upon the chemical environment. In previous
papers (Cole and Allison, 1930–31; 1931–32) probable effects of the
structure and potential of very reactive groups as well as the structure
and potential of less reactive groups of the stimulating compound
have been considered in a general way. Homologous series of organic
compounds have been and are being studied because of the better
known variation of their chemical and physical properties as the
number of CH₃ groups increases. Such a study it is hoped will lay
the foundation for an investigation of more complex series of related
compounds.

It has been assumed that the response measured is a function of the
intensity of stimulation of the receptor interface, and that whether the
reaction is best related to an interfacial tension change, or to a re-
distribution of charged particles at the surface, or to some unknown
chemical situation in the receptor itself, it is, however, reversible and
may eventually be related to the chemical nature of the substance
which initiated a series of events in the heterogeneous system. For
example, stimulation by the normal aliphatic alcohols has been related
to the length of the carbon chain. It may be assumed that an energy

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change is produced at the receptor interface which is initiated by the
spreading of alcohol molecules at the interface, and that this spreading
is influenced by the chemical nature of the receptor as well as by its
chemical environment. A shift in the ionic equilibrium at the inter-
face would also follow. Although other factors may eventually be
identified as playing roles in stimulation by alcohol, including the
efficiency of the neuro-muscular mechanism of the animal itself, it is
clear that the reaction measured may be correlated with the non-polar
portion of the molecule and its power to initiate energy changes at an
interface. The effect of the polar group in the alcohol molecules
appears constant, and it may be said to function primarily as an
orienting group. In fact, the effect of an organic molecule upon the
tension of an air-water interface may be considered as a measure of
the potential and structure of the non-polar group as well as a measure
of the orienting properties of the polar group which in turn are related
to its chemical nature. However, as in the case of reactions at any
interface, the structure and chemical nature of the receptor may
modify or change the relationships between the efficiency of a series of
compounds. For example in the isomeric alcohols, stearic hindrance
may modify the reaction, and in the aliphatic acids the polar group
may play a more or less active role in shifting the dynamic equilibrium
at the receptor interface. The data presented in this report deal with
the latter problem.

Methods

Fresh water sunfish, *Eupomotis gibbosus* Linnaeus, of uniform size (approxi-
mately 3–4 cm. in length) were kept in separate containers in spring water. The
apparatus and technique used in the experiments were similar to those used for
the studies on the catfish *Schilbeodes* (Cole and Allison, 1931–32). An individual
was transferred to the reaction chamber so that the flow of spring water (100 cc. ±
5 cc. per minute) passed in the antero-posterior direction in reference to the fish.
Except for a few exceptions, 10 minutes were allowed for adaptation of the fish to
the container. The flow of spring water was then turned off and the experimental
solution turned on at the same rate. The reaction observed consisted in cessation
of mouth movements or in a marked change in rate which may be best expressed
by the word "gulping." The reaction time was measured with a stop-watch to
within 0.1 seconds. The response is definite and characteristic and may or may
not be accompanied by bodily movements. The latter were not timed. The
animal was then thoroughly washed with spring water and transferred for several
minutes to a large dish containing fresh spring water before returning it to the original container. Eight fish were tested in this way in succession and at no time were more than two reactions taken on one fish on 1 day. Usually 1 to 2 hours elapsed before the fish was used for a second experiment. The spring water and experimental solution were brought to the same temperature by passing them through a Pyrex coil 4 meters long immersed in a water bath kept at 18.0 ± 0.1°C. The acids were purified and fresh solutions were made in spring water the day they were to be used. The (H⁺) was measured with the quinhydrone electrode. The pH of the spring water varied from pH 6.4 to 6.8.

In his study on sensory activation of the earthworm by alkalies Crozier (1917-18) reduced the average reaction time by a constant which gave him a measure of the true retraction time. The significance of the data on the sunfish was increased by subtracting 3 seconds from the average reaction time for each concentration. The value subtracted here may be considered as an approximation of the shortest reaction time possible without producing toxic effects under the above experimental conditions. "Reaction time," hereafter will refer to the corrected values.

RESULTS AND DISCUSSION

In a previous paper (Cole and Allison, 1931-32) it was pointed out that a receptor which is susceptible to a slight change in (H⁺) of its chemical environment might appear equally affected by inorganic or organic acids if the concentrations needed were so low that other disturbances related to the activity of the anion, or to the non-polar portion of the organic molecule, had little or no effect. The statement was also made that in the case of normal aliphatic acids, "as the length of the carbon chain increases the non-polar portion of the molecule might begin to play a stimulatory rôle and with the higher members of the series, it might predominate." It is also evident that certain receptors under proper conditions might react to a combination of such forces. A study was made on stimulation by hydrochloric acid in the catfish Schilbeodes, and the conclusion was reached that the reactions observed could be correlated with the potential of the cation. The data obtained on the sunfish lead to the same conclusion, except that this animal is much less susceptible to a change in the (H⁺) of its environment and shows also (as might be predicted) more susceptibility to the changes initiated by the non-polar portion of an n aliphatic acid molecule.

It is apparent (Table I, Fig. 1) that when reaction time is related to the pH of the solution in spring water some other factor than the
(H⁺) is involved in the initiation of the response to the N aliphatic acids. With increasing length of carbon chain these acids become more efficient as stimulating agents. However, if the logarithm of the concentration is related to the logarithm of the rate of reaction (Fig. 2) formic acid appears much more effective than should be the case if the reaction involved was primarily one that could be related to the tendency of the organic acids to concentrate at an air-water or an air-oil interface. The ability of the acid to change the (H⁺) of the spring water may be used as one measure of its polar nature. As would be expected, hydrochloric acid is more effective in this respect than formic

**TABLE I**

Reaction times of the sunfish, *Eupomotis*, to different concentrations of hydrochloric acid and of six N aliphatic acids. The number of reactions is 16 except those indicated by a * where it is 15.

<table>
<thead>
<tr>
<th>Acid concentration- molarity</th>
<th>pH</th>
<th>Mean reaction time (sec)</th>
<th>pH</th>
<th>Mean reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0049</td>
<td>2.48</td>
<td>2.42</td>
<td>0.184</td>
<td>2.48</td>
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<tr>
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<td>2.48</td>
<td>0.143</td>
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<td>0.127</td>
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<td>2.97</td>
<td>4.36</td>
<td>0.227</td>
<td>2.97</td>
</tr>
<tr>
<td>0.00196</td>
<td>3.20</td>
<td>5.60</td>
<td>0.429</td>
<td>3.20</td>
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<tr>
<td>Formic acid</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00149</td>
<td>2.92</td>
<td>2.56</td>
<td>0.179</td>
<td>2.92</td>
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<tr>
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<td>2.43</td>
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<tr>
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<td>Acetic acid</td>
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<td>0.241</td>
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<td>4.45</td>
<td>6.66</td>
<td>0.916</td>
<td>4.45</td>
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</tbody>
</table>

\[ p.e. = \pm 0.8453 \frac{\sum (+v)}{n \sqrt{n - 1}} \]
acid, which in turn is more so than the other members of the homologous series. If the concentrations in moles per liter of acetic, propionic, butyric, valeric, and caproic acids used in this work are plotted against the pH of the spring water solution, a smooth curve may be drawn which will include all points. Formic acid is not out of orderly position if it is assumed that the response measured may be initiated not only by the reactivity of the non-polar group but also by that of the polar group. This assumption is supported by the position of the formic acid curve between the hydrochloric acid curve and that for acetic acid shown in Fig. 1 and by its position in Fig. 2.

It is important to note that the slope of the formic acid curve
(Fig. 2) is less than for the others which results in a differing relationship between this acid and the other members when equally effective concentrations are related to the length of the carbon chain. At the higher concentrations formic acid is less efficient when compared with the other members than it is at lower concentrations. Stimulation of the rock barnacle, *Balanus balanoides*, by the salts of the $N$ aliphatic acids (Cole, 1931–32) shows a similar relationship between sodium formate and the other members of the homologous series. With increasing concentration sodium formate becomes less and less efficient when compared with sodium acetate, sodium propionate, etc. This variation may be interpreted to mean that as the concentration of

![Graph showing the logarithm of the rate of stimulation of the sunfish, *Eupomatis*, to hydrochloric, formic (F), acetic (A), propionic (P), butyric (B), valeric (V), and caproic (C) acids against the logarithm of the concentration ($N \times 10^3$) (data in Table I).]
formic acid is increased the effect upon the receptor interface becomes more of the nature of that produced by the higher members of the series.

It is concluded then that the dominant factor in stimulation of the sunfish by the \( n \) aliphatic acids may be correlated with the non-polar nature of a portion of the molecule, but that it is necessary to consider the higher potential of the polar group of formic acid to account satisfactorily for its position in the series. Experiments made by Crozier (1917–18) on the stimulating efficiency of the fatty acids on the earthworm, and his interpretation thereof, support this argument.

The reliability of the data which has been presented may be tested in part by utilizing the principles of reaction variation which have been developed by Crozier and Pincus (1931–32, and papers quoted therein). Although it was impossible to be certain that the population of fish selected for these experiments was homogeneous, the probable errors of the mean reaction times do show a consistent relationship to

![Graph](image-url)

**FIG. 3.** A mass plot of the probable errors, expressed as percentage of the mean reaction time; against the reaction time. (See text.) \( n = 16 \) except for three points which are indicated by horizontal lines where it is 15.
the variable pH. A series of curves similar to those shown in Fig. 1 are obtained when the probable errors are related to the pH of the solution of each acid. When the probable errors, expressed as percentage of the reaction time, are plotted en masse against the reaction time, a horizontal relationship between the two values appears which means that the per cent variation is independent of the change in the chemical environment to which the animal responded.

SUMMARY

1. The reaction of the sunfish, *Eupomotis gibbosus*, to different concentrations of hydrochloric acid and of the first six members of the $N$ aliphatic acids has been studied.
2. The stimulating efficiency of hydrochloric acid may best be related to the concentration of hydrogen ions produced by that acid.
3. The stimulating efficiency of the $N$ aliphatic acids may best be correlated with the non-polar nature of a portion of the molecule, but it is necessary to consider the higher potential of the polar group of formic acid to account satisfactorily for its position in the series.
4. When equally effective concentrations of the $N$ aliphatic acids are compared, formic acid is more effective at lower concentrations than at higher.
5. Per cent variation in response appears to be independent of the chemical environment to which the animal responded.

REFERENCES