STIMULATION BY THE MINERAL ACIDS, HYDROCHLORIC, SULFURIC, AND NITRIC, IN THE SUNFISH EUPOMOTIS

By WILLIAM H. COLE* and J. B. ALLISON

(From the Department of Physiology and Biochemistry, Rutgers University, New Brunswick)

(Accepted for publication, October 17, 1932)

I

In previous studies on chemical stimulation of aquatic animals it has been assumed that the response measured is a function of the intensity of stimulation of the receptor surface, and that the response may be correlated with the nature of the stimulating substance which initiates a series of energy changes in the heterogeneous system consisting of the chemical environment of the animal and the receptors concerned. The last event in the series activates the neuromuscular mechanism, the efficiency of which is assumed constant in any individual under ordinary conditions. Adaptation and injury must be absent and the energy changes at the receptor surface must be reversible, since the responses are easily reproducible.

The original energy change is initiated by the alteration of the ionic or molecular equilibrium and strength of the animal's chemical environment. Stimulation by the primary aliphatic alcohols is evidently a direct effect upon the receptor surface brought about by the addition of alcohol molecules to the environment and by their specific orientation in relation to that surface (Cole and Allison, 1930–31). Since the polar hydroxyl group ($-$OH) in the alcohols appears to be constant, the predominate factor in stimulation is the field of force around the R group. Similarly, stimulation by the normal primary aliphatic acids in the sunfish (Allison, 1931–32) may be best correlated with the non-polar portion of the molecule, except for the first member of the series where it is necessary to consider the higher potential of the

---

* A part of the expenses of this investigation was met by a grant from the National Research Council for 1932.

polar group as playing a significant rôle. Again, stimulation by the salts of the normal primary aliphatic acids in the barnacle appears to be chiefly correlated with the non-polar portion of the molecule (Cole, 1931–32). On the other hand, stimulation by the same acids in the fresh-water catfish *Schilbeodes* is dependent upon the potential of the polar group, or upon the ability of that group to change the (H^+) of the environment.[1] Such a dependence might be predicted because of the great susceptibility of the catfish to slight changes in (H^+) (Cole and Allison, 1931–32). As the length of the carbon chain increases, the non-polar portion of the molecule may begin to play a rôle.

In any complete series of polar organic molecules it is likely that stimulation may be traced ultimately to the activity of the polar group, or to the field of force around the non-polar group, or to both. One or the other factor may predominate, or each may play a significant rôle. It is logical therefore to consider separately the field of force around the radical *R* and the potential of the polar group such as $-OH$, $-COOH$, $-CHO$, $-NH_2$, etc. If the stimulating substance is dissociated into ions which in themselves may be considered entirely polar, such as are produced from inorganic compounds, it would also be logical to consider separately the fields of force around each ion.

II

Further experiments have been done on the sunfish *Eupomotis gibbosus*, to determine whether disturbances in the chemical environment initiated by the anions of the strong inorganic acids, hydrochloric, sulfuric, and nitric, would alter the relationship between (H^+) and reaction time as demonstrated for hydrochloric acid alone (Allison, 1931–32); the procedure used was the same as that already described. Features of the method are the constant rate of flow of solution and spring water over the fish, allowance of ample time for recovery of the fish between trials, thereby preventing any adaptation, and measurement of the (H^+) of each solution by the quinhydrone electrode. Two series of animals were used over a period of 6 months.

---

[1] Unpublished results obtained in 1930.

In one series two observations were made on each of eight fish for each concentration ($n = 16$), and in the other series two observations on each of ten fish ($n=20$), resulting in 628 observations on eighteen fish. The data for hydrochloric acid where $n=16$ are taken from Allison (1931–32). Freshly collected fish responded more quickly than those well adapted to laboratory conditions. Relatively, however, the relation between rate of response and concentration was the same for each group. By adding 1.25 seconds to the reaction times of freshly collected fish the two sets of data become coincident. As before, the actual reaction times have been corrected by subtracting 5.0 seconds, which is considered to be the theoretical shortest possible reaction time obtainable from any individual. Confirmation of this value is found by extrapolating the (H^+) where $y = 0$, corresponding to a pH of 1.88 (see Fig. 2). In the preliminary tests it was regularly noted that a pH of 2.0 or less was injurious to the fish and caused an increase in reaction time, indicating that other processes were occurring besides stimulation.

III

When an acid, such as hydrochloric, is added to the chemical environment of the fish, not only is the (H^+) changed but also the (Cl^-), and a new equilibrium is established throughout the whole system. The degree of shift in the ionic and molecular equilibrium of the system may be related to the effective concentration of one of the ions or the other. If the animal's response is a function of the (H^+) alone, then no difference would be observed between the stimulating efficiency of different acids used to alter the concentration of that ion. If, however, the specific nature of the anion plays the predominate rôle, then each of the acids would give different results. For the sunfish the data secured show clearly that stimulation by hydrochloric, sulfuric, and nitric acids is primarily dependent upon the (H^+) within the range tested (Table I). In Fig. 1 are plotted thirty-four average reaction times as related to the (H^+) produced in the environment by adding hydrochloric, sulfuric, and nitric acids. The smooth curve is drawn through coordinates calculated from the equation of the line in Fig. 2. That line was laid off through the middle of a parallel band of points with a

TABLE I

Corrected Reaction Times of Sunfish to Different Hydrogen Ion Concentrations in Solutions of Hydrochloric, Sulfuric, and Nitric Acids Made in Spring Water

(H^+) conc. $\times 10^4$	$RT-5$	P.E._{RT-5}
	Hydrochloric	
	sec.	
6.31*	5.60	0.429
10.00	4.75	0.296
10.72*	4.36	0.227
13.49*	3.46	0.127
16.22	4.31	0.344
21.88	3.57	0.218
25.71	3.43	0.240
31.63*	2.48	0.143
33.12*	2.42	0.184
35.49	2.50	0.276
	Sulfuric	
7.08	5.88	0.575
9.55*	4.72	0.351
12.89	4.63	0.286
13.81*	4.24†	0.226
20.90	3.21	0.269
22.91*	3.37†	0.149
24.55*	2.96	0.196
30.91*	2.18	0.129
31.63	2.21	0.166
	Nitric	
5.62	5.73	0.362
6.31	5.51†	0.285
6.46	5.18†	0.246
6.60	5.57	0.416
10.48	4.50	0.241
10.97*	4.83†	0.207
16.22	4.27†	0.190
16.99	3.95	0.235
18.20*	4.07†	0.198
21.88	3.76	0.213
21.88	3.52	0.210
23.99*	3.20†	0.152
25.12	3.26†	0.175
26.31	2.13	0.137
37.16	2.52	0.166

$n = 20$ except in cases indicated by *, where $n = 16$. The reaction times for freshly collected, non-adapted fish, indicated by †, have been increased by 1.25 sec. to make them coincident with the other data.

uniform distribution on each side. There is evidently no specific effect of the anions on the reaction time. It is often assumed, and probably correctly, that the hydrogen ion exerts a more or less direct effect upon the living system by shifting the (H^+) one way or

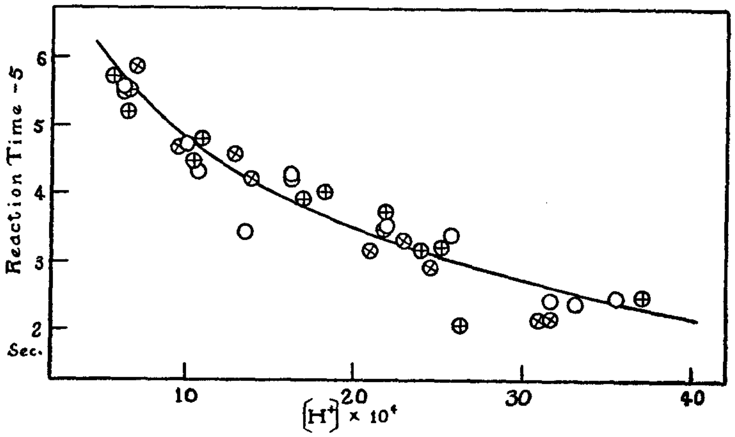

FIG. 1. The exponential relationship between corrected reaction times and (H^+), as shown by the sunfish when stimulated by various concentrations of hydrochloric (○), sulfuric (⊗), and nitric (⊕) acids. The smooth curve is drawn through coordinates calculated from the equation of the line in Fig. 2.

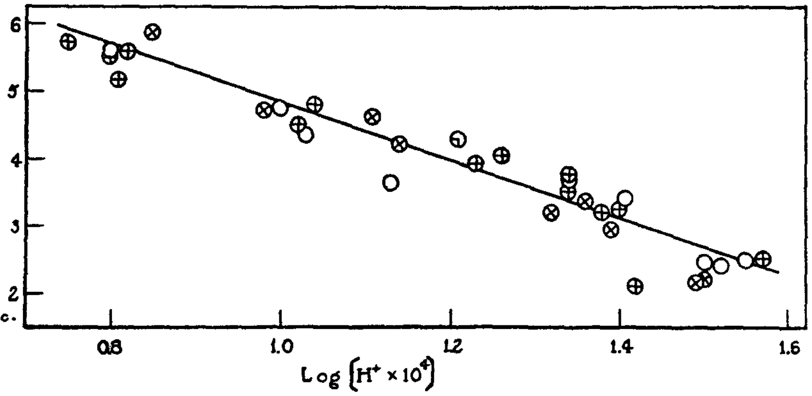

FIG. 2. Logarithmic plot of the same data as in Fig. 1. The linear relationship may be expressed by the equation: $RT - 5 = -4.3 \log (H^+ \times 10^4) + 9.118$. (The symbol (⊕) represents coincident points for hydrochloric and nitric acids.)

the other from the isoelectric point of some constituent in that system. Whether or not such an effect is present in the sunfish cannot yet be proved, but it is interesting to note that as the (H^+) increases geometrically the reaction time increases arithmetically. In other words,

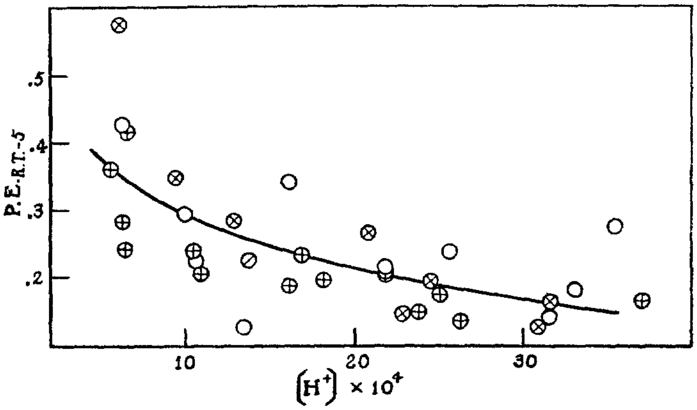

FIG. 3. The exponential relationship between the probable errors of the corrected reaction times and (H^+). Same symbols as in Fig. 1. The smooth curve is drawn through coordinates calculated from the equations of the lines in Figs. 2 and 4.

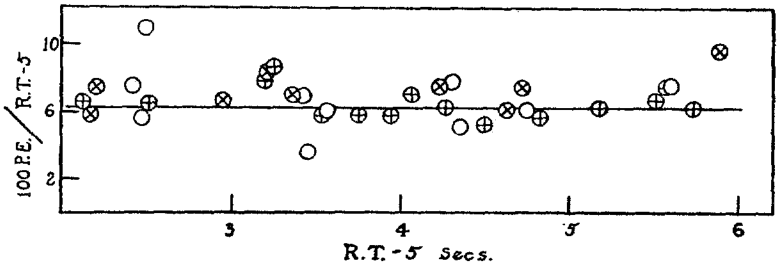

FIG. 4. The linear relationship between the percentage probable errors of the corrected reaction times and the corrected reaction times. Symbols as in Fig. 1. $y = 6.2$.

the reaction time is a logarithmic function of the (H^+). Any disturbance of the equilibrium between receptor and the environment produced by the chloride, sulfate, and nitrate ions must be itself measured by the (H^+) of the solution.

Variations in the response as shown in Figs. 1 and 2 are assumed to be distributed uniformly about the theoretical lines. It is known that those variations are not due entirely to observational error or to experimental technique. They must be primarily intrinsic variations of the mechanism that determines the animal's response to the stimulus. If the efficiency of that mechanism does not change over the range of (H^+) tested, then the percentage variation of the mean reaction times should be constant when the number of observations and the number of animals are constant. That this is true is shown in Fig. 4. Percentage variation in reaction time is independent of the (H^+) over the range tested. To show that an exponential relationship also exists between (H^+) and the latitude of variation a smooth curve was drawn in Fig. 3 through coordinates calculated from the equations of the lines in Figs. 2 and 4. The general trend of the actual probable errors of the reaction times is thus indicated. Again no differences between the effects of hydrochloric, sulfuric, and nitric acids are noticeable.

In connection with the variations in response above noted, it should be emphasized that the data presented were obtained at different times over a period of 6 months, and on individuals obtained at different times from three different localities. Some of the fish were well adapted to laboratory conditions, but a few were used immediately after collection. No difference however in the exponential relationship between (H^+) and reaction time was apparent. It was only necessary to recognize the lower threshold for the freshly collected fish, and to make the proper correction in the reaction times. The percentage variation in response was the same for both groups.

SUMMARY

1. The stimulating efficiency of hydrochloric, sulfuric, and nitric acids has been measured in the sunfish *Eupomotis gibbosus*, by a method which reduces experimental errors to a minimum.

2. The results show that stimulation by these acids is primarily dependent upon the (H^+) produced in the animal's aquatic environment, and that the reaction time is a logarithmic function of the (H^+) within the range tested expressed by the equation: $(RT - 5) = -4.3 \log (H^+ \times 10^4) + 9.118$.

3. Any effect of the chloride, sulfate, and nitrate ions must itself be measured by the (H^+).

4. Variation in the reaction time is also a logarithmic function of the (H^+), and the percentage variation is independent of the (H^+) over the range tested.

5. Freshly collected fish show a lower threshold for stimulation as determined by the (H^+) than do fish adapted to laboratory conditions, but relatively the reaction times of the two groups are the same.

CITATIONS

Allison, J. B., *J. Gen. Physiol.*, 1931–32, **15,** 621.

Cole, W. H., *J. Gen. Physiol.*, 1931–32, **15,** 611.

Cole, W. H., and Allison, J. B., *J. Gen. Physiol.*, 1930–31, **14,** 71; 1931–32, **15,** 119.