STIMULATION OF FUNDULUS BY HYDROCHLORIC AND FATTY ACIDS IN FRESH WATER, AND BY FATTY ACIDS, MINERAL ACIDS, AND THE SODIUM SALTS OF MINERAL ACIDS IN SEA WATER

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INTRODUCTION

Stimulation of aquatic animals by substances dissolved in the medium will be interpretable when the rôles played both by the environment and by the receptive mechanism are understood. Stimulating forces originating in the environment are effective only when the receptive mechanism is so adjusted that an interaction between environment and receptors is possible. In any one animal the range of receptive processes is strictly limited and it is impossible to change it significantly by experimental procedure without causing "abnormal" reception. Similarly any marked change in the environment is equally disastrous to normal reception in the majority of animals. However, there are a few animals which can live equally well in quite different media. Of these the killifish *Fundulus heteroclitus* is a good example, since it can live in fresh, brackish, or salt water without showing abnormal behavior. If stimulation by the same substance in different media should result in different responses a correlation between the specific environments and the receptive mechanism would be indicated; but if no difference in response appeared, it would be suggestive of an independence of reception upon the change of general environment. This animal has therefore been used to test the stimulating effectiveness of certain acids and salts in fresh and salt water. Hydrochloric and five normal aliphatic acids (acetic, propionic, butyric, valeric, and caproic) were tested in fresh water, while the same acids plus sulfuric and nitric, as well as the sodium salts of the mineral acids, were tested in salt water.[1]

The experimental procedure in all cases was the same as that described for the sunfish (Allison, 1931–32), except that tap water was used instead of spring water for the fresh water tests, and that the rate of flow for the salt water tests was 250 cc. instead of 100 cc. per minute.

When fresh or salt water flows over *Fundulus*[2] under constant experimental conditions the rate of mouth movements and opercular movements remains fairly constant. Occasionally there may be movements of the fins or body and variations in the respiratory movements, but such behavior only temporarily interferes with the tests. When an acid solution replaces fresh or salt water the fish responds by closure of the mouth and opercula. When inorganic salts are used, the response is more often a gaping of the mouth or gulping, followed by closure. The response is usually very definite and easily recognized. The time between turning on the solution and the response is measured and designated the reaction time. During the night and other periods when not being used, the fish are returned to their individual aquaria where feeding occurs. The animals may be used repeatedly for several weeks (8 to 12) and continue to give the same reaction times (± 5 per cent) for the same solutions.

The reaction time is a measure of the response to stimulation. For the acids it is correlated with the concentration of the hydrogen ion[3] and for the salts with the normal concentration. In the case of the fatty acids another factor appears to play a rôle in stimulation, namely, the field of force around the non-polar group in the molecule. This factor increases as the number of CH_2 groups increases,

[1] The experiments with salt water were done at the Mt. Desert Island Biological Laboratory during the summers of 1932 and 1933. Preliminary reports of the results were published in the *Bull. Mt. Desert Island Biol. Lab.*, 1933, 27; 1934, 33.

[2] In any group of *Fundulus* there are always some individuals which cannot be used for this type of experiment, because they move about incessantly. The majority, however, quickly become acclimated to the experimental dish and remain quiet for hours unless stimulated.

[3] The pH of the solutions both in fresh and salt water was measured by the quinhydrone electrode and calculated from the equation:

$$\mathrm{pH} = \frac{-E + 0.4529 + 0.00002t}{0.00019832T}$$

Check determinations by the hydrogen electrode showed variations from the quinhydrone values which were no larger than the errors of measurement. Hydrogen ion concentrations were then calculated from Sørensen's equation:

$$\mathrm{pH} = \log \frac{1}{C_{\mathrm{H}}}$$

and may be indirectly measured by the hydrogen ion concentration. The intensity of stimulation by each acid, as measured by the reaction time, appears to be some function of the activity of the hydrogen ion. There are several ways of correlating these two variables, but they are not all equally suggestive of an interpretation. In choosing a method of representation there are certain points to be noted. First, the reaction time must be corrected to give a value which is clearly related to the stimulus. As measured by the stop-watch the reaction time includes a certain minimum time required by (1) the passage of the afferent nervous impulse to the central nervous system; (2) internuncial coordination; (3) the passage of the efferent impulse to the effectors; and (4) the processes of muscular contraction which close the mouth and opercula. As long as the intensity and frequency of stimulation are low enough to avoid all secondary effects, such as adaptation, narcosis, toxicity, and the like, that time is constant. It should therefore be subtracted from that observed reaction time, since it is not correlated with the stimulus. Furthermore a certain time is lost before the solution comes into contact with the receptors, and its magnitude depends upon the rate of flow of solution over the fish and upon the volume of the container. This should also be subtracted from the reaction time. For the experiments in fresh water with a flow of 100 cc. per minute the combined correction factor was 4 seconds, as determined by the maximum rate of reaction at different rates of flow. For the salt water tests with flows of 250 cc. per minute, only 2 seconds were needed to correct the observed reaction time. Secondly, it is desirable to use *rate of reaction* instead of reaction time in seconds, since rate, considered as a function of some variable, is easier to interpret than measured time. Finally the data are so expressed that when one variable is plotted against the other a linear relationship appears.

Experiments in Fresh Water

Using *Fundulus* in fresh water, hydrochloric, acetic, propionic, butyric, valeric, and caproic acids were tested at several concentrations each. As with the catfish *Schilbeodes* (Cole and Allison, 1931–32), a single *Fundulus* gives as reliable results as several individuals averaged together, and makes unnecessary the correction for individual variations in thresholds. Table I summarizes all the data secured from a single fish. In Fig. 1 the logarithm of rate of reaction is plotted against logarithm of (H^+), and the lines are drawn from the following equation[4] which relates rate of reaction with (H^+):

$$\log \frac{100}{RT - 4} = 0.665\,[1 - 0.5^{(1 + 0.7n)}]\,\log\,((H^+) \times 10^6) + 0.56 \qquad (1)$$

[4] The equation was derived from the line drawn through the HCl points, so that it would represent fair agreement with the other points and approach a limiting value when $n = 6$.

TABLE I

Reaction Times of Fundulus to Acids in Fresh Water

Temperature: 18 ± 0.2°C. Rate of flow: 100 cc. per minute. n = 10. The values in Column 3 were calculated from equation (1).

pH	Observed $RT - 4$	Calculated $RT - 4$	Probable error of $RT - 4$*
	Hydrochloric		
	sec.	*sec.*	
3.02	2.72	2.81	0.1060
3.17	3.30	3.09	0.1776
3.44	3.80	3.88	0.2312
4.00	6.00	5.95	0.5471
4.68	9.30	10.02	0.7106
	Acetic		
3.74	2.69	2.49	0.1996
3.90	3.79	2.97	0.2473
4.10	4.42	3.68	0.2893
4.33	5.67	4.69	0.3934
4.64	7.61	6.52	0.6294
5.10	14.78	10.61	0.8922
	Propionic		
3.75	2.20	1.69	0.0903
3.92	2.73	2.08	0.1326
4.16	3.30	2.81	0.1269
4.65	4.70	5.16	0.2015
5.06	8.04	8.58	0.4850
5.21	13.65	10.33	0.5386
	Butyric		
4.10	2.23	2.11	0.1286
4.21	2.65	2.44	0.1100
4.38	2.74	3.08	0.1410
4.88	6.49	6.05	0.4641
4.98	5.40	6.93	0.2087
5.29	9.88	10.54	0.6926
	Valeric		
4.20	2.31	2.13	0.1714
4.34	2.99	2.60	0.1553
4.79	4.03	4.92	0.1438
4.93	6.10	6.02	0.7219
5.35	7.61	10.93	0.5104
	Caproic		
4.52	2.94	3.16	0.1714
4.85	4.58	5.12	0.1375

* P.E. = $\pm 0.8453 \dfrac{\Sigma(+v)}{n\sqrt{n-1}}$

where n = number of CH_2 groups in the acid molecule. (For hydrochloric acid n = 0.) For each acid the equation may be written in the simplified form:

$$RT - 4 = \frac{1}{K(H^+)^b}, \tag{2}$$

where K and b are constants which fix the position of each line. There are definite experimental limits within which these equations describe

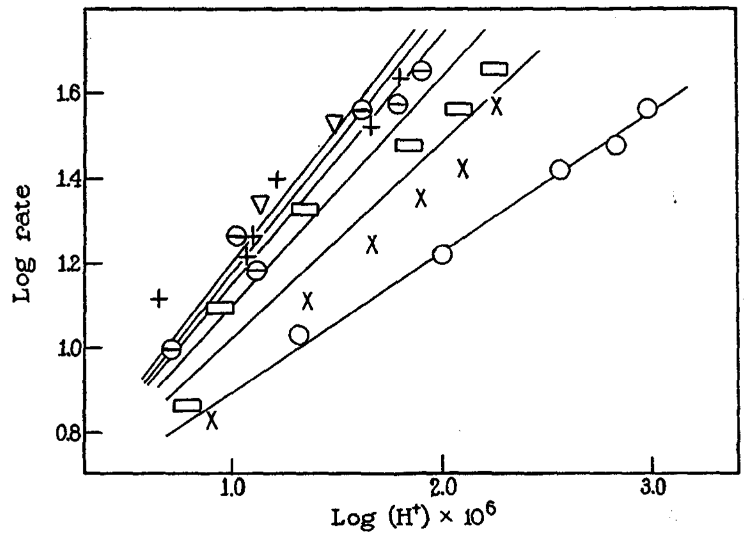

FIG. 1. Log of rate of reaction $\left(\text{where rate} = \frac{100}{RT - 4}\right)$ plotted against log $(H^+) \times 10^6$ for stimulation of *Fundulus* in fresh water by the following acids: HCl (○), acetic (×), propionic (▭), butyric (⊖), valeric (+), caproic (▽). (See text for further description.)

the process of stimulation of *Fundulus* by acids in fresh water. These limits are defined by the plot. Since the lines all intersect where reaction time = 27.5 seconds and the $(H^+) = 1 \times 10^{-6}$ N, it is obvious that extrapolation of the equations below these points would have no experimental justification. The common intersection further shows that stimulation by any of the acids may be correlated solely with the potential of the hydrogen ion at the lowest (H^+) value. Although the lines drawn from equation (1) do not fit some of the plotted points very well, the following interpretation would still be justified even though slightly different lines or no lines at all were drawn.

Stimulation by HCl is correlated with the effective hydrogen ion concentration. Stimulation by the fatty acids, as their concentration increases, is correlated with two factors: the effective hydrogen ion concentration, and the potential of the non-polar group in the molecules. The latter potential increases by a fixed amount as each CH_2 group is added to the molecule beginning with acetic acid. However, not all of this added energy is available for stimulation, since as the number of CH_2 groups increases the stimulating effect increases by smaller and smaller amounts, and gradually approaches a maximum value, as shown by equation (1), where the limiting slope approaches 0.665. This situation differs from stimulation by alcohols, where each CH_2 group increases effectiveness by a constant amount (Cole and Allison, 1930–31).

The results correspond qualitatively with those obtained on the sunfish (Allison, 1931–32; Cole and Allison, 1932–33 *a*). Both *Eupomotis* and *Fundulus* react in the same way to mineral and fatty acids. In each fish stimulation by the mineral acids may be correlated with the effective hydrogen ion concentration, and stimulation by the fatty acids with length of the carbon chain. However, there are quantitative differences. The sunfish has a higher threshold for stimulation by hydrochloric acid than *Fundulus* and the mathematical statements describing the relationship between (H^+) and rate of reaction also differ for the two fish. Stimulation by the fatty acids in *Eupomotis* is correlated primarily with the field of force around the non-polar group, which is not true for *Fundulus*. Furthermore in the sunfish a limiting stimulating value determined by the number of CH_2 groups in the molecule is not reached so quickly as in *Fundulus*. The difference in behavior must be due to differences in the receptive mechanism. Although the equilibrium is shifted in both cases by a change in (H^+), a shift of stimulating proportions is reached at a lower (H^+) in *Fundulus* than in *Eupomotis*, which means that the dynamic equilibrium between receptors and environment is different in the two fish. The simplest assumption to make is that the chemical natures of the receptors differ. Such a difference would also account for the different behavior of the two fish towards stimulation by the fatty acids.

In *Fundulus* the interface might become saturated with fatty acids lower in the series than in the sunfish, so that addition of more CH_2 groups to the molecule produces no further increase in stimulation, while in *Eupomotis* saturation occurs only with the higher acids in the series, allowing increased stimulation as more CH_2 groups are added. In both fish the concentration of fatty acids at the receptor surface, as determined by the length of the carbon chain and by the composition of the receptor surface, would increase the activity of the hydrogen ion at the interface beyond that of the medium as a whole. If it is further assumed that the only rôle of the non-polar group in the fatty acids is to concentrate the acids at the receptor interface, then equal rates of reaction in one fish would be produced by equal hydrogen ion concentrations at the surface, regardless of the acid used, even though the hydrogen ion concentrations of the media as a whole were quite different for the different acid solutions. However, since intensity of stimulation is always measured by a change in hydrogen ion concentration of the media, stimulation by the fatty acids must be correlated wholly or in part with a force which is not measured in terms of primary valence.

Experiments in Sea Water

(*a*) *Mineral Acids*

With *Fundulus* adapted to sea water the stimulating efficiencies of HCl, H_2SO_4, and HNO_3 were tested at several concentrations. Ten observations on each of two fish were made at each concentration. The two sets of data were so similar that they were averaged (see Table II). In Fig. 2 the logarithm of the rate is plotted against the logarithm of the (H^+), revealing a linear relationship over the experimental range. Per cent variation[5] is essentially constant, indicating no change in the mechanism of the stimulation process. The three mineral acids evidently enter into the reaction in equivalent amounts. Stimulation by these acids must therefore be correlated with the so called chemical forces of primary valence which can be measured by the hydrogen ion concentration. The line in Fig. 2 is much like the

[5] Per cent variation $= \frac{100 \text{ P.E.}}{RT - 2}$. The average per cent variation for the mineral acids in sea water = 6.7. (*Cf.* Allison, 1931–32, and Cole and Allison, 1932–33 *a.*)

TABLE II

Reaction Times of Fundulus to Mineral Acids in Sea Water

Temperature: 17.7 ± 0.2°C. Flow: 250 cc. per minute. n = 20, 10 on each of two fish.

pH	$RT - 2$	Probable error*	pH	$RT - 2$	Probable error	pH	$RT - 2$	Probable error
Hydrochloric acid			Sulfuric acid			Nitric acid		
	sec.			*sec.*			*sec.*	
2.70	1.66	0.1148	2.68	1.76	0.1241	2.70	1.46	0.0712
3.12	2.36	0.1556	2.89	2.06	0.1226	2.90	2.01	0.0942
3.18	3.24	0.2487	3.24	2.23	0.0976	3.27	2.64	0.1272
3.34	2.67	0.1336	3.53	3.24	0.2594	3.61	2.97	0.1083
3.52	2.65	0.1883	3.85	3.14	0.1754	3.81	3.23	0.1827
3.60	2.77	0.1480	4.16	4.22	0.2924	3.95	3.82	0.2479
3.79	3.30	0.2510	4.46	5.76	0.3714	4.14	4.29	0.2188
3.93	3.79	0.2842	4.53	4.61	0.4097	4.49	5.16	0.3787
4.00	3.57	0.2960	4.72	5.15	0.3039	4.94	6.16	0.4286
4.08	4.71	0.2941	5.08	7.93	0.5787	5.19	9.13	0.5059
4.34	5.01	0.3464	5.10	6.29	0.4689	5.28	8.79	0.5528
4.42	7.23	0.4912	5.22	11.66	0.8513	5.47	9.32	0.6046
4.49	5.95	0.5550	5.41	10.24	0.4450	5.50	11.47	0.8760
4.77	5.57	0.5651	5.50	10.26	0.5549	5.73	10.23	0.4987
4.85	7.37	0.5758	5.60	11.85	0.6900	5.75	11.67	0.6933
5.00	5.88	0.5919	5.87	14.24	0.8995	5.85	14.23	0.9199
5.18	8.81	0.6488						
5.23	10.16	0.7912						
5.25	12.60	1.1939						
5.70	14.47	1.4681						
5.80	11.50	0.8056						
5.80	14.08	1.0389						

* P.E. $= \pm 0.8453 \dfrac{\Sigma(+v)}{n\sqrt{n-1}}$

HCl line in Fig. 1, and can be described by the same equation (Equation 2) with slightly different constants, as follows:

$$\text{for HCl in fresh water: } RT - 4 = \frac{1}{3.59(H^+)^{0.3325}} \quad (3)$$

$$\text{for HCl, } H_2SO_4\text{, and } HNO_3 \text{ in sea water: } RT - 2 = \frac{1}{3.716(H^+)^{0.292}} \quad (4)$$

Both equations are subject to about the same experimental limits. Such close agreement indicates that a change from fresh to sea water does not alter the type of reaction at the receptor interface when mineral acids are added to the environment of *Fundulus*. By increasing the (H^+) of either fresh or sea water with mineral acids, the established equilibrium existing between receptors and environment is so shifted that the same response occurs.

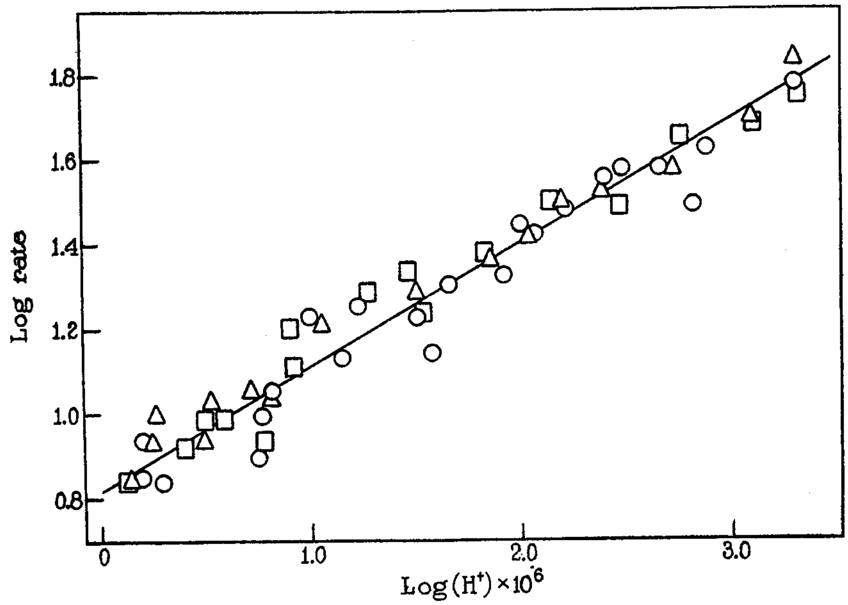

FIG. 2. Log of rate of reaction $\left(\text{where rate} = \frac{100}{RT - 2}\right)$ plotted against log ((H^+) $\times 10^6$) for stimulation of *Fundulus* in sea water by HCl (○), H_2SO_4 (□), and HNO_3 (△). (See text for further description.)

A year previously qualitative data were secured for stimulation by HCl and by the fatty acids (acetic, propionic, butyric, valeric, and caproic) in sea water, and are presented in Fig. 3. Although the threshold for stimulation by HCl was slightly lower, the slope of the line is the same as that for HCl in Fig. 2, showing excellent agreement with the later tests. Here again evidence exists that the fatty acids are more effective than the mineral acids because of the presence of CH_2 groups in the molecules. Although there are not sufficient data to justify a quantitative treatment, the difference in spread of the lines for fatty acids in Fig. 1 and Fig. 3 is considered significant, and not as due to experimental or other systematic errors. In fact, a difference might be expected because over the whole experimental range it was necessary to use higher concentrations of fatty acids in sea water than in fresh water in order to produce the same pH. For example, 0.0004 N propionic acid in sea water changed the pH from 8.2 to 7.4, while in fresh water it changed the pH from 8.0 to 5.2. A lower concentration

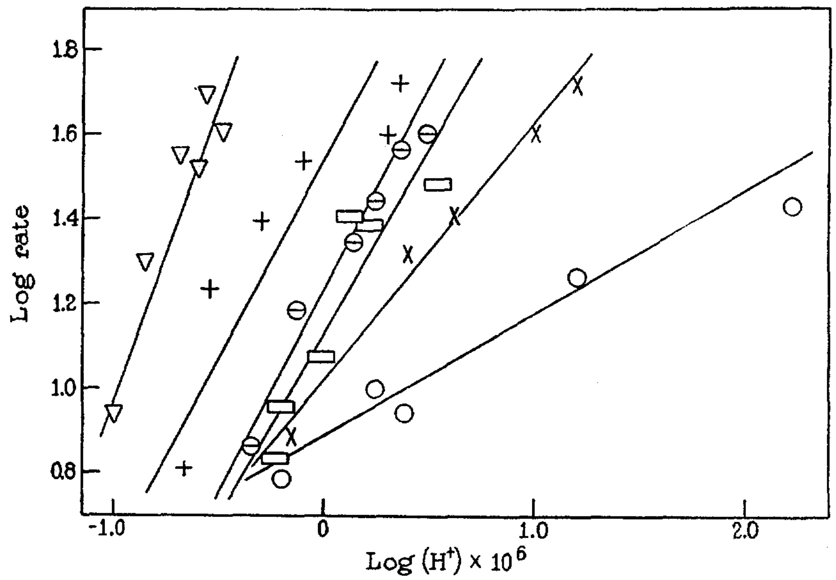

FIG. 3. Log of rate of reaction $\left(\text{where rate} = \frac{100}{RT - 2}\right)$ plotted against log ((H+) × 10⁶) for stimulation of *Fundulus* by the following acids: HCl (○), acetic (×), propionic (▭), butyric (⊖), valeric (+), caproic (▽). (See text for further description.)

of free fatty acid and a higher concentration of the salts of the fatty acids therefore exist in the sea water solutions than in the fresh water solutions. Furthermore, there is a greater change in CO_2 pressure in the sea water solutions than in fresh water, since sea water is buffered mostly by bicarbonates. Since little difference was found between the stimulating efficiencies of the mineral acids in fresh and in sea water, a change in CO_2 pressure is probably not the reason for the different effects of the fatty acids in the two environments. However, part of this difference must be explained by the formation of higher concentrations of the fatty acid anion in sea water than in fresh water. The tendency of this anion to alter the complex ionic equilibrium at or near the receptor interface in sea water has been recognized in the interpretation of experiments with the barnacle (Cole, 1931–32; Cole and Allison, 1932–33b).

TABLE III

Reaction Times of Fundulus to Inorganic Salts in Sea Water

Temperature: 17.7 ± 0.2°C. Flow: 250 cc. per minute. n = 20, 10 on each of two fish. Concentration normal = normal concentration of salt added to sea water.

Concentration normal	$RT - 2$	Probable error	Concentration normal	$RT - 2$	Probable error	Concentration normal	$RT - 2$	Probable error
Sodium nitrate			Sodium chloride			Sodium sulfate		
	sec.			*sec.*			*sec.*	
0.08	19.74	1.0459	0.08	16.91	1.0825	0.08	17.46	1.2627
0.085	18.12	1.5865	0.085	16.53	1.3863	0.09	13.61	1.0326
0.09	17.94	1.3477	0.09	14.60	1.0163	0.10	10.45	0.4977
0.095	13.91	1.3316	0.095	16.77	1.1956	0.11	9.49	0.4458
0.10	11.62	0.4090	0.10	10.88	0.5479	0.12	9.52	0.7352
0.105	12.69	0.9380	0.105	12.81	0.8540	0.13	7.87	0.4901
0.11	10.57	0.4498	0.11	13.28	0.8194	0.14	5.92	0.2916
0.115	8.55	0.6945	0.115	8.43	0.4430	0.16	5.03	0.2918
0.12	8.70	0.3578	0.12	8.93	0.5916	0.18	5.23	0.2989
0.125	9.97	0.8812	0.125	8.91	0.5947			
0.13	8.23	0.5231	0.13	9.29	1.0025			
0.135	9.61	0.9159	0.135	9.80	0.6529			
0.14	6.07	0.3076	0.14	8.72	0.6412			
0.145	6.15	0.5837	0.145	8.93	0.3677			
0.15	6.56	0.4235	0.15	7.82	0.8446			
0.155	4.93	0.6720	0.155	7.83	0.6110			
0.16	4.95	0.3117	0.16	6.45	0.6895			
0.165	5.46	0.5710	0.165	5.72	0.4269			
0.17	4.85	0.2594	0.17	6.80	0.6601			
0.175	5.35	0.5563	0.175	6.71	0.7402			
0.18	4.80	0.3158	0.18	4.69	0.3372			
			0.19	5.16	0.5062			

(*b*) *Inorganic Salts*

Tests were also made with the sodium salts of HCl, H_2SO_4, and HNO_3; the data are collected and summarized in the same way as for the acids (see Table III and Fig. 4). The pH of the salt solutions was the same as that of sea water. The responses of the fish to a change in salt concentration of the sea water were not as regular as they were to a change in (H^+), and often consisted of a gaping of the mouth or gulping followed by closure. Sometimes no easily recognizable response occurred when the salt solutions were used, especially at the lower concentrations. Therefore a few "no reactions" were interspersed with the obvious reactions. The former were not included in the analysis of the data. This difference in behavior of the fish to-

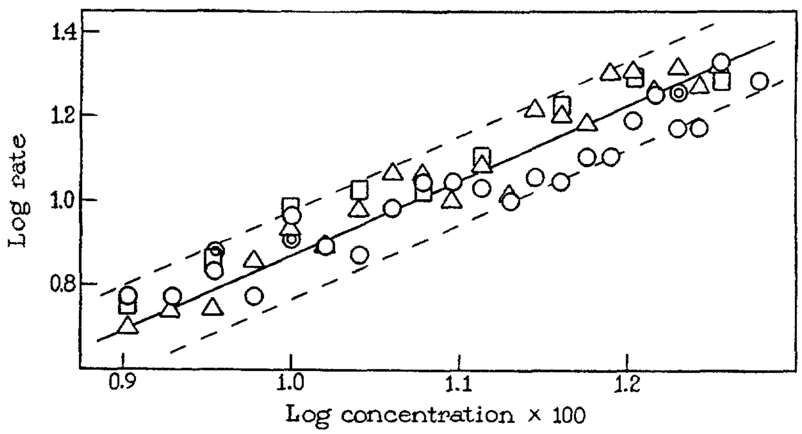

FIG. 4. Log rate of reaction $\left(\text{where rate} = \frac{100}{RT - 2}\right)$ plotted against log concentration × 100 for stimulation of *Fundulus* by NaCl (○), Na_2SO_4 (□), and $NaNO_3$ (△) in sea water. The three points marked by two concentric circles (◎) are the averages of twenty observations on each of the three salts by a different observer using a different individual *Fundulus* at a different rate of flow (100 cc. per minute). To correct these reaction times 4 seconds were subtracted instead of 2.

wards stimulation by the salts and acids may indicate a difference in the mechanism of the stimulation processes. The average percentage variation for the salts was 7.5, which is slightly higher than for the acids, but all of the points lie within two parallel lines defining the scatter. The average line may be represented by the following equation within the experimental limits:

$$RT - 2 = \frac{1}{4.498\ C^{1.78}}, \qquad (5)$$

where C represents equivalent concentrations of the salts added to sea water. Check tests for each of the salts indicated by concentric circles in Fig. 4 were made by another observer on a single fish adapted to a different experimental set-up with a lower rate of flow (100 cc. per minute). There is no apparent break in the relationship stated by equation (5), since the per cent variation is independent of the change in concentration.

Although much higher concentrations of the anions were used in the salt solutions than in the acid solutions, there is no evident effect which can be correlated with difference in the polarity of these ions. Sodium chloride, sodium sulfate, and sodium nitrate enter into the reaction in equivalent amounts, so that surface forces which might alter such a stoichiometric relationship play no rôle. Stimulation by these salts as well as by the mineral acids can therefore be correlated with the forces of primary valence. However, the stimulating efficiency of the sodium salts measured by threshold concentrations is much less than for the acids. The greater threshold concentration of the salts might be expected since the fish is adapted to a relatively high concentration of sodium ions in sea water. The efficiency of the reaction in stimulation by salts and by acids may also be measured by the slopes of the lines in Fig. 4 and Fig. 2. The greater the slope the greater the efficiency of the reaction over the experimental range. The concentration exponent in equation (5), which is the slope of the line, is 1.78 while the exponent in equation (3) for the mineral acids is 0.292. Therefore after the respective threshold concentrations have been reached the sodium salts are more effective as stimulating agents than the mineral acids.

SUMMARY

1. *Fundulus heteroclitus* was found to be a reliable experimental animal for studies on chemical stimulation in either fresh or sea water.

2. The response of *Fundulus* to hydrochloric, acetic, propionic, butyric, valeric, and caproic acids was determined in fresh water, while the same acids plus sulfuric and nitric, as well as the sodium salts of the mineral acids, were tested in sea water.

3. Stimulation of *Fundulus* by hydrochloric acid in fresh water is correlated with the effective hydrogen ion concentration. Stimula-

tion by the *n*-aliphatic acids in the same environment is correlated with two factors, the effective hydrogen ion concentration and the potential of the non-polar group in the molecule. However, as the number of CH_2 groups increases the stimulating effect increases by smaller and smaller amounts, approaching a maximum value.

4. Stimulation of *Fundulus* by hydrochloric, sulfuric, and nitric acids in sea water is correlated with the forces of primary valence which in turn are correlated with the change in hydrogen ion concentration of the sea water. The *n*-aliphatic acids increase in stimulating efficiency in sea water as the length of the carbon chain increases, but a limiting value is not reached as soon as in fresh water.

5. Only a slight difference in stimulation by hydrochloric acid is found in sea water and in fresh water. However, there is a significant difference in stimulation by the fatty acids in fresh and in sea water, which is partly explained by the different buffering capacities of the two media. It is to be noted that in the same environment two different fish, *Fundulus* and *Eupomotis*, give different results, while the same fish (*Fundulus*) in two different environments responds similarly to mineral acids but differently to fatty acids. These results illustrate that stimulation is a function of the interaction between environment and receptors, and that each is important in determining the response.

6. Stimulation by sodium chloride, nitrate, and sulfate is correlated with equivalent concentrations of the salts added to sea water, or with the forces of primary valence. Although the threshold for stimulation by the salts is considerably higher than for the acids, the efficiency of stimulation by the salts is greater.

CITATIONS

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