

## STIMULATION BY MINERAL AND FATTY ACIDS IN THE BARNACLE *BALANUS BALANOIDES*\*

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A previous study on the stimulating efficiency of the salts of the first seven normal aliphatic acids at constant pH ( $= 8.1 \pm 0.15$ ), on the barnacle *Balanus balanoides*, indicated increasing effectiveness as the length of the carbon chain increased (Cole, 1931-32). The data were presented graphically by plotting per cent closure, or area under plots of percentage difference in number open at the end of 8 minutes, against molar concentration. As a first approximation smooth curves were drawn through the points, and the assumption was made that a normal distribution of thresholds existed in the population of animals used. It was pointed out that formate seemed to be out of place in the order of effectiveness if 50 per cent closure or less (instead of 60 per cent or more) were taken as the criterion of response.

A complementary study of stimulation by the fatty acids has been made by allowing the pH to vary according to the amount of acid added to sea water. In addition, three mineral acids have been tested. Any effect due to the alteration in the  $(H^+)$  should be identified by this method. Such an effect has been demonstrated, and a more detailed analysis of the response as related to the  $(H^+)$  and to the length of the carbon chain has been made possible.

The experimental procedure used was similar to that previously described, except in the making of solutions. The following acids at from 8 to 14 concentrations each were used: hydrochloric, 0.0002 to 0.0025 N; sulfuric, 0.0004 to 0.0025 N;

\* The experiments were done at the Mt. Desert Island Biological Laboratory during the summer of 1932.

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nitric, 0.0002 to 0.00253 N; formic, 0.0002 to 0.002 N; acetic, 0.0002 to 0.003 N; propionic, 0.0004 to 0.002 N; butyric, 0.0002 to 0.002 N; valeric, 0.0004 to 0.002 N; caproic, 0.0002 to 0.002 N; and heptylic, 0.0002 to 0.0014 N. The ( $H^+$ ) varied from  $0.32 \times 10^{-7}$  to  $58.89 \times 10^{-7}$ .

18 liters of each solution were made by adding the desired amount of acid to fresh sea water. The mixture was thoroughly shaken and left standing until the ( $H^+$ ) as measured by the quinhydrone electrode became relatively constant. This interval was  $45 \pm 15$  minutes. Since sea water is buffered chiefly by bicarbonates, the addition of an acid to the system liberates carbonic acid and the change in pH is an approximate measure of the amount of carbonic acid produced when all of the acid added has reacted with the bicarbonates. Under these conditions the pH of sea water will change by about the same amount when equivalent concentrations of strong and weak acids are added. If the same concentrations, however, are made up in carbonate-free sea water adjusted to a pH of 8.3, the pH of the solution will change in accordance with the strengths of the acids added. Furthermore, if more acid is added than can react with the bicarbonates, then the pH of untreated sea water similarly changes in accordance with the strength of the acid used.

Each solution was divided into three parts and each part was tested by a different observer<sup>1</sup> on a different group of barnacles, totaling 222 individual barnacles. The rate of flow of solutions over the animals was  $250 \pm 15$  cc. per minute, and the temperature was  $17.0 \pm 0.3^\circ C$ . The number of regularly active animals (Cole, 1932) was recorded at 1 minute intervals and plotted as ordinates against time in minutes. The three plots for each solution were so nearly alike that averaging of the ordinates was justified. From the combined data the percentage of animals closing during each minute interval was calculated as a percentage on the basis of the number of animals open at the end of the interval as compared to the number open at the beginning of the test.

Two methods of treating the data were used. The first was similar to that employed for the fatty acid salts (Cole, 1931-32). Per cent closure was plotted against ( $H^+$ ) and smooth curves were drawn through the points. A family of exponential curves rising sharply from the origin, becoming asymptotic at progressively greater ( $H^+$ ), and revealing a graded series of constants, was displayed. The relationships showed that in the lower range all the acids were about equally effective as stimulating agents, but that as the ( $H^+$ ) increased effectiveness increased with the number of  $CH_2$  groups. Very little difference between the mineral acids and the first three fatty acids was

<sup>1</sup> The writers are indebted to Miss Helen H. Smith and Mr. Irwin W. Sizer for assistance in making observations and in many other ways.

apparent, but beginning with butyric acid the spread of the curves decreased and steadily became less through heptylic. The stimulating effect of the fatty acids is clearly related to the disturbances in the environment which are produced both by the non-polar portion of the molecule and by the change in  $(H^+)$ . Compared with fatty acid salts at constant pH, the free acids are about ten times more efficient as stimulating agents for the barnacle.

For preliminary interpretation the following development is helpful. If the amount of change in the number of animals open ( $c$ ) as compared with that of the intensity of stimulation ( $x$ ) is proportional to  $(c)$  then the following equation may be written

$$\frac{dc}{dx} = -kc. \quad (1)$$

Upon integration, equation (1) becomes:

$$\frac{c}{c_0} = e^{-kx}, \quad (2)$$

where  $c_0$  is the original number of animals open. To show the relationship between per cent closure and intensity of stimulation, equation (2) may be revised as follows:

$$\text{Per cent closure} = 100 - 110e^{-kx}. \quad (3)$$

The total disturbance to which the animals respond within a fixed time is proportional to two forces, one related to the change in the  $(H^+)$  and the other to the field of force around the anion of the acid added to the environment. Both forces may be measured in terms of  $(H^+)$ , and the following expression may be developed from (3) to fit the data obtained at the end of a 4 minute interval:

$$\text{Per cent closure} = 100 - 100e^{-0.1z + (0.003125)2^n(z-0.4)} \quad (4)$$

where  $z$  is  $(H^+) \times 10^7$  and  $n$  is the number of carbon atoms (if present) in the anion of the acid.

A family of exponential curves, derived from equation (4), is reproduced in Fig. 1. The lowest curve, where  $n = 0$ , approximately describes the relationship between per cent closure at the end of 4 minutes and the change in  $(H^+)$  produced by adding one of the mineral

acids. The curve for formic acid, where  $n = 1$  (not shown in Fig. 1) is almost coincident with the one for mineral acids, lying only slightly above the latter.<sup>2</sup> No great difference between the stimulating efficiencies of the mineral acids and of formic acid appears, which means that equivalent concentrations of the anions enter into the equilibrium

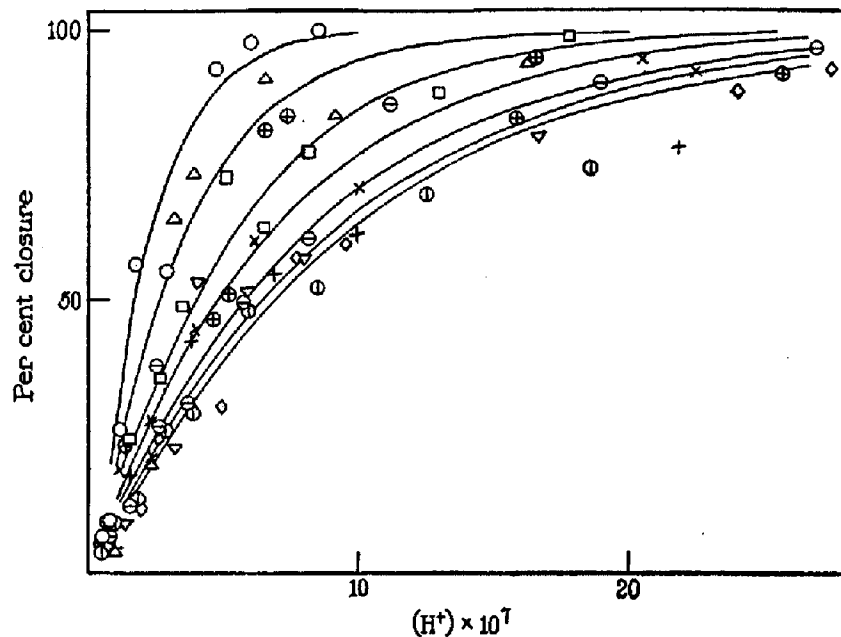


FIG. 1. A series of exponential curves derived from equation (4) in the text. The plotted points are average values obtained by using the following acids: HCl ( $\oplus$ ),  $\text{H}_2\text{SO}_4$  ( $\nabla$ ),  $\text{HNO}_3$  (+), formic ( $\odot$ ), acetic ( $\times$ ), propionic ( $\diamond$ ), butyric ( $\ominus$ ), valeric ( $\square$ ), caproic ( $\triangle$ ), and heptylic ( $\circ$ ) as stimulating agents on the barnacle. Temperature =  $17.0 \pm 0.3^\circ\text{C}$ .

of the system stoichiometrically. Structure of the anion appears to play no rôle in the case of these acids.

The next six curves, for acetic, propionic, butyric, valeric, caproic, and heptylic acids, represent the effect of increasing  $n$  from 2 to 7 inclusive. By adding these acids to sea water there is produced an

<sup>2</sup> The ordinates for the formic acid curve are on the average less than 1 per cent (of closure) higher than those for the mineral acid curve.

additional effect correlated with the structure of the anion and quantitatively determined by the number of  $\text{CH}_2$  groups. When the carbon chain contains 5 atoms or more this additional effect becomes approximately equal to the hydrogen ion effect. As would be expected, the difference between the members of the series becomes more and more marked as the concentration increases.

Deviations of the data from the theoretical interpretation just presented are noticeable, and appear to be greater than the experimental errors in measurement. A more complete analysis demands that these deviations be considered as significant. The second method of treating the data was to plot per cent closure against  $\log (\text{H}^+)$ , giving equal weight to each point.<sup>3</sup> Unmistakable evidence of a compound sigmoid relationship appeared for each acid, as illustrated in Fig. 2. Although indications of the sigmoid character of the curves were noticed when  $(\text{H}^+)$  was plotted as abscissae, they became marked in the  $\log (\text{H}^+)$  plots. The amount of excitation, as measured by per cent closure, can therefore be considered more accurately as a logarithmic function of the  $(\text{H}^+)$ . Now if a normal distribution of one kind of receptors within the population were involved, an S-shaped curve should more clearly describe the data than the shape of the curves shown in Fig. 1. If more than one group of receptors, or if more than one group of thresholds, exists in the population, then the plots should indicate it by the number of summated sigmoid curves. The importance of such an analysis has been emphasized by Hecht (1927-28) and by Crozier and Pincus (1929-30, 1931-32) for other cases. When the differential of the curves,  $\frac{\Delta \text{ per cent closure}}{\Delta \log [(\text{H}^+) \times 10^8]}$ , is related to  $\log (\text{H}^+) \times 10^8$ , a significant series of trimodal distribution figures appears (Fig. 3). It may be assumed that each figure is the resultant of three approximately symmetrical distribution curves, indicating in the population of animals (or in any one individual) the existence of three chemoreceptor groups differing in mean thresholds. Overlapping of the groups is apparent, and the per cent closure is determined additively as the  $(\text{H}^+)$  increases.

It is impossible to know at this time the exact number of groups,

<sup>3</sup>  $\log (\text{H}^+)$  was used in order (1) to plot conveniently the highest  $(\text{H}^+)$ , and (2) to spread out the points near the lowest  $(\text{H}^+)$  values.

whether they are morphological or functional units, and whether they are groups of receptors or of individuals. If the multimodal sensitivity is due to forces other than those correlated directly with the

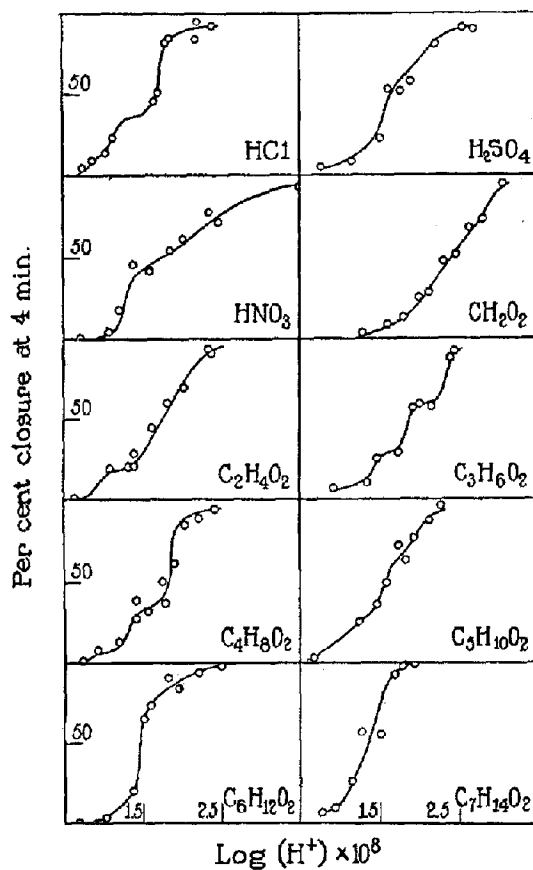


FIG. 2. Plots showing the compound sigmoid relationship between per cent closure and the  $\log (H^+) \times 10^8$  for hydrochloric, sulfuric, nitric, formic, acetic, propionic, butyric, valeric, caproic, and heptylic acids when used as stimulating agents on the barnacle. Temperature =  $17.0 \pm 0.3^\circ\text{C}$ .

stimulating agents then the system is a fluctuating one, and the phenomenon could not be correlated alone with the stimulating agent but also with other variables such as age factors and a fluctuating environment. Since exactly the same population of individuals was

employed for each acid, and since some checks were made on the reliability of former results over an interval of 10 weeks, it is believed that the three or more groups represent receptor groups with differen-

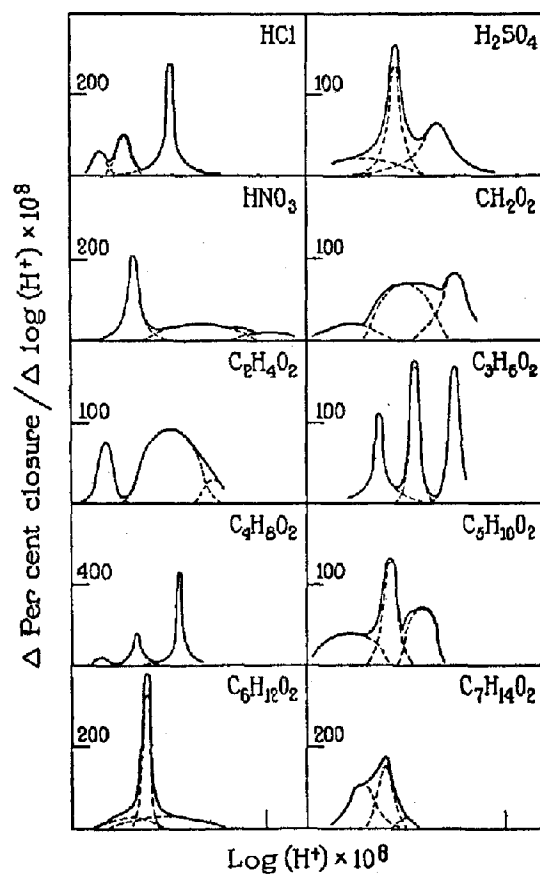


FIG. 3. Differential plots of the corresponding curves in Fig. 2. The scale along the  $x$  axis is identical to that in Fig. 2. Each figure is separated into three symmetrical distribution curves. For further explanation see text.

tial sensitivity. Information concerning the correlates of such differential sensitivity may be obtained from further investigation.

At any given  $(H^+)$  the stimulating forces attributable to the hydrogen ion are constant, and any difference in sensitivity of cor-

responding groups among the ten acids may be due to the difference in the field of force around the anion. The per cent closure obtained at a given ( $H^+$ ) is the result of the summation of the excitation of all three groups for each acid as presented in Fig. 2. If the degree of closure (from 60 to 80 per cent inclusive) is considered a measure of stimulation, equally effective hydrogen ion concentrations for the ten acids may be calculated and plotted against the type of anion (for example, the number of carbon atoms in the molecule), indicating the following order of stimulating efficiency for the fatty acids: heptylic > caproic > valeric > butyric = acetic > propionic = formic; and for the mineral acids: hydrochloric > sulfuric > nitric.

The second method of analyzing the data leads to essentially the same conclusion as the first one does; namely, that stimulation by mineral and fatty acids in the barnacle is correlated with two closely related factors, the ( $H^+$ ) or the potential of the cation, and the field of force around the anion. New information is revealed indicating the presence of three or more groups of receptors and their differential sensitivities, which explains why the first analysis is incomplete, why it does not show differences between the effects of the chloride, sulfate, and nitrate anions, and why the order of effectiveness among the fatty acids shows some variation from what might have been expected.

#### SUMMARY

1. Stimulation in the rock barnacle *Balanus balanoides* by hydrochloric, sulfuric, and nitric acids, and by the first seven members of the normal aliphatic acid series has been studied. The hydrogen ion concentrations of the solutions tested varied from  $3.2 \times 10^{-8}$  to  $5.889 \times 10^{-8}$ . The criterion of response was percentage closure in groups of individuals, recorded at 1 minute intervals until maximum closure occurred.

2. The intensity of stimulation by these acids is proportional to the effects of two forces, one related to the change in the ( $H^+$ ), and the other to the field of force around the anion of the acid added to the environment.

3. A preliminary interpretation of the results led to the develop-



ment of the following expression which fits approximately the data obtained at the end of 4 minutes:

$$\text{Per cent closure} = 100 - 100e^{-0.1z + (0.003125)2^n(z-0.4)}$$

where  $z$  is the  $(H^+) \times 10^7$  and  $n$  is the number of carbon atoms (if present) in the anion of the acid. This equation assumes that the anions of the mineral acids enter into the reaction stoichiometrically, and emphasizes the difference in the fields of force around the anion of the fatty acids, a difference which is correlated with the length of the carbon chain.

4. A further analysis of the data revealed the presence of three or more receptor groups which appeared to be differentially affected by forces originating from the anions of the acids.

5. The order of stimulating efficiency for the mineral acids was found to be:  $HCl > H_2SO_4 > HNO_3$ .

6. The order of stimulating efficiency for the fatty acids was found to be: heptylic > caproic > valeric > butyric = acetic > propionic = formic.

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