

STIMULATION BY THE SALTS OF THE NORMAL ALIPHATIC
ACIDS IN THE ROCK BARNACLE *BALANUS*
BALANOIDES

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EXPERIMENTAL

As it has been demonstrated that closure of the valves, and changes in the cirral rhythm, of the rock barnacle *Balanus balanoides* are reliable criteria of stimulation (Cole, 1932), experiments were made with the salts of the normal aliphatic acids.¹ With a population of 137 animals under constant environmental conditions, the relation between the percentage of closed animals at successive 1 minute intervals and the concentrations of the salts has been determined. The animals were approximately 3 years of age, varying in basal diameter from 8 to 15 mm., and in height from 5 to 12 mm. They were seated on a flat stone, evenly distributed over its surface, and were kept continuously in running sea water for 7 weeks. Temperature was controlled thermostatically at $17.2 \pm 0.2^\circ\text{C}$.; the rate of flow was 250 ± 25 cc. per minute; artificial illumination was constant, and vibrational stimuli were reduced to a negligible minimum. The apparatus was so arranged that the sea water flowing over the animals could be shut off and replaced by the experimental solution at the same rate of flow and temperature, as described in previous papers (Cole, 1928-29; Cole and Allison, 1930-31; 1931-32). The average number of regularly active animals (those showing their normal rhythmic rate) previous to each test was 67, or about 50 per cent of the total. Of the others about 10 per cent were irregular, and about 23 per cent were closed.

From four to nine different concentrations of each of the following acids made up in 3 liters of sea water were used at the same pH as sea water: formic, acetic, propionic, butyric, valeric, caproic, and heptylic, involving 158 tests.² The pH

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¹ The experiments were done at the Mount Desert Island Biological Laboratory.

² The acids were repurified from Eastman products of the highest purity and their physical constants checked against those given in the International Critical Tables.

was adjusted by adding small amounts of strong NaOH solutions made in distilled water. Since the pH of sea water was always close to 8.1 (± 0.15), the acids were immediately neutralized, forming solutions of the corresponding sodium salts. The number of regularly active animals was recorded at 1 minute intervals for from 8 to 12 minutes, after which no further change in the number occurred. The animals were then thoroughly rinsed in excess sea water and allowed a rest period of 30 minutes before the next test. Reproducible results indicated the complete absence of any adaptive or harmful effects. At the end of the experiments the animals appeared to be in as good a condition as at the beginning, as judged by the rates of cirral movement (Cole, 1932).

RESULTS

For analyzing the data obtained it was assumed that there was a normal distribution of thresholds among the animals previous to each test. During the first interval the animals with the lowest threshold closed, during the last interval those with the highest threshold closed, and during the intervening intervals closure occurred progressively according to the intermediate thresholds. The percentage of animals closing in any interval, therefore, became a measure of the effectiveness of the solution tested, and was calculated on the basis of the number of animals open at the end as compared to the number open at the beginning of the test. Distribution plots of the percentage differences for successive 2 minute intervals were constructed for each concentration of each salt, and the areas enclosed by the plots were calculated graphically. The presence of a symmetrical mode in each case with a decrease in percentage on either side supports the assumption that the thresholds are distributed normally in the population. By plotting the areas against concentration a graphical representation of the effectiveness of the solutions was obtained. Such plots were made for 2, 4, 6, and 8 minute intervals, and in spite of minor differences appeared to be essentially alike, especially the 4, 6, and 8 minute plots. Fig. 1 reproduces the 8 minute plot which will serve as a typical illustration, and furnishes the evidence for the following discussion. Similar relationships appeared when total per cent closure at the end of the successive 2 minute intervals was plotted against concentration.

For the seven heptylate solutions (from 0.0008 to 0.004 M) maximum closure was always obtained at the end of from 30 to 60 seconds. In

spite of continued exposure to the solutions recovery (opening of the valves) began immediately and continued until the end of the 2nd or 3rd minute, when about 50 per cent of the animals had opened again. From then on the rate of closure followed the same course as for the other salts. A similar effect, but less marked, was noted also in the

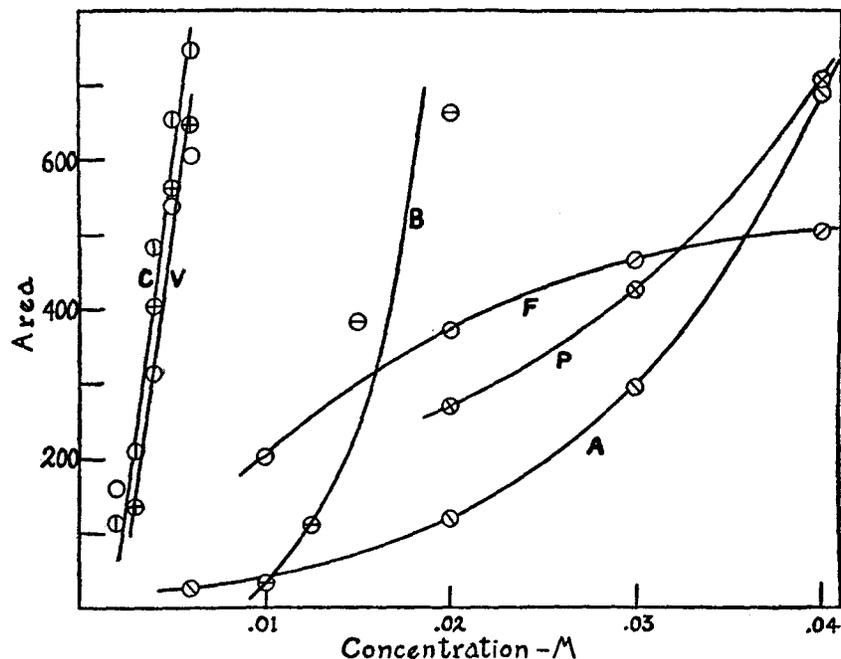


FIG. 1. Effectiveness of solutions of fatty acid salts as stimulating agents on the barnacle, as measured by the areas under the plots of percentage difference in the number of animals closed at the beginning of the test and at the end of 8 minutes, plotted against molar concentration. *F* = sodium formate; *A* = sodium acetate; *P* = sodium propionate; *B* = sodium butyrate; *V* = sodium valerate; *C* = sodium caproate. The open circles represent the values of sodium heptylate, for which no line is drawn. Temperature, $17.2 \pm 0.2^\circ\text{C}$.

higher concentrations of valerate (from 0.003 to 0.01 M). It was absent entirely in the more dilute solutions (0.002 and 0.0025 M) of valerate and in all solutions of the other salts. Although no interpretation of this rapid recovery is offered, its regular occurrence deserves mention.

Fig. 1 shows that effectiveness of the solutions increases with the concentration for all the salts, but that the ratios of effects at increasing concentrations differ for each salt. Omitting the special case of formate, the other salts show an increasing effectiveness with length of carbon chain. The curves become progressively steeper, until the relationship is practically linear for the last three salts. Heptylate appears to be no more effective than caproate, however. Spacing of the plots along the x axis is not logarithmic, indicating that the ratio of effectiveness in respect to length of carbon chain is not a simple exponential function as was found for the alcohols (Cole and Allison, 1930-31). The plot further shows that as the concentration of acetate and propionate increases, their effectiveness converges in such a way that at about 0.04 M they are equal. The formate curve is different from the others in being concave to the x axis, and in having a much lower gradient. For butyrate the effectiveness increases remarkably more than for either acetate or propionate; and for the valerate, caproate, and heptylate the increase is still more marked. In other words, the range of concentrations within which there is a graded response correlated with concentration is largest for formate and steadily decreases up to valerate. Qualitatively this is similar to the results obtained from the alcohols.

Since the area plotted along the y axis is a measure of the effectiveness of each solution, it is possible to read from the plot the concentrations which are equally effective. From the plot of Fig. 1, and from the plots of total percentage closure against concentration, equally effective concentrations were interpolated for areas of 400 and 600, and for 40 per cent, 50 per cent, and 60 per cent closure. Excepting the 60 per cent closure effect, the interpolated values for any given effect were nearly constant, and they were therefore averaged. Fig. 2 shows the plot of these averages against the number of carbon atoms in the chain (continuous line), and the concentrations necessary to produce 60 per cent closure (dotted line). The concentrations for the first effect are: formate 0.0183; acetate 0.032; propionate 0.028; butyrate 0.0153; valerate 0.0044; caproate 0.0037 and heptylate 0.0037 M. In general it may be said that to produce a given effect which will not exceed 50 per cent closure, formate is more effective than acetate and propionate, and only slightly less effective than

butyrate, but considerably less effective than any salt higher in the series. Beginning with acetate the effectiveness increases quite regularly with length of carbon chain up to and including valerate. If a greater effect is selected as a criterion (*i.e.*, 60 per cent closure) formate also falls into the series, being much less effective than acetate

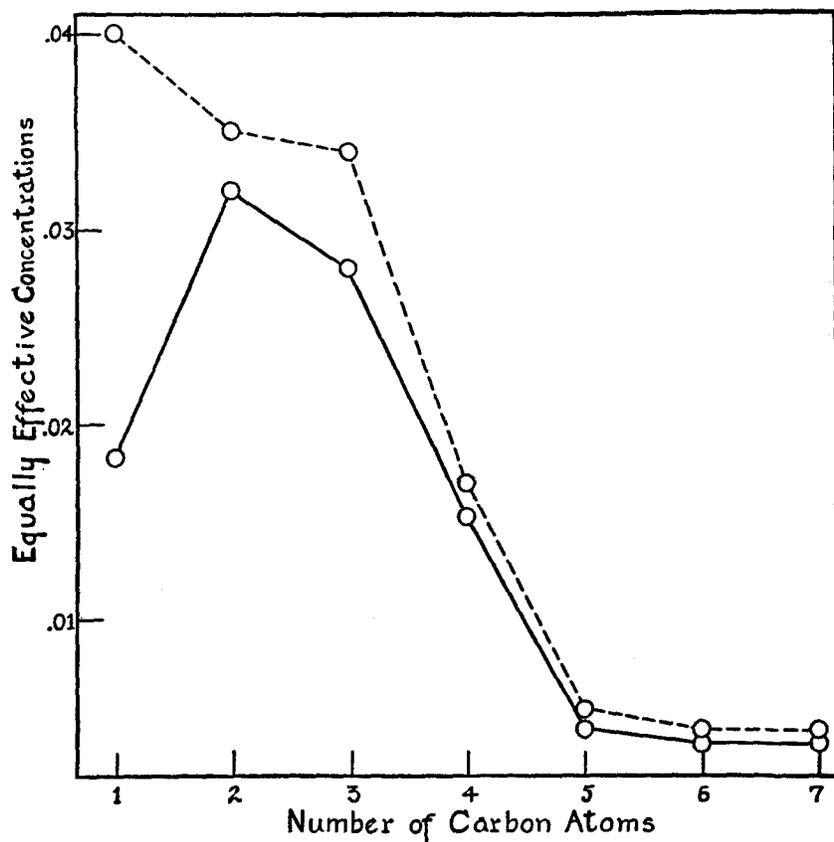


FIG. 2. Concentrations of the seven salt solutions which were equally effective plotted against the number of carbon atoms in the respective molecules. The solid line is drawn through the average concentrations as interpolated from the plots of area (400 and 600) *vs.* concentration and the plots of 40 per cent and 50 per cent closure for the 8 minute interval. The dotted line connects the concentrations as interpolated from the 60 per cent closure plot for the 8 minute interval.

(dotted line, Fig. 2). In measuring the effect of these salts upon the barnacle therefore special attention must be given to the criterion indicative of the effect. A relationship similar to each of those mentioned above has been previously demonstrated for sea urchin eggs by Loeb (1908-09), and for the earthworm by Crozier (1916).

Measurements of the surface tension at an air-sea water interface were made by the Harkins' drop weight method (Harkins and Brown, 1919; 1929), at $17.2 \pm 0.2^\circ\text{C}$., of sea water, of the equally effective

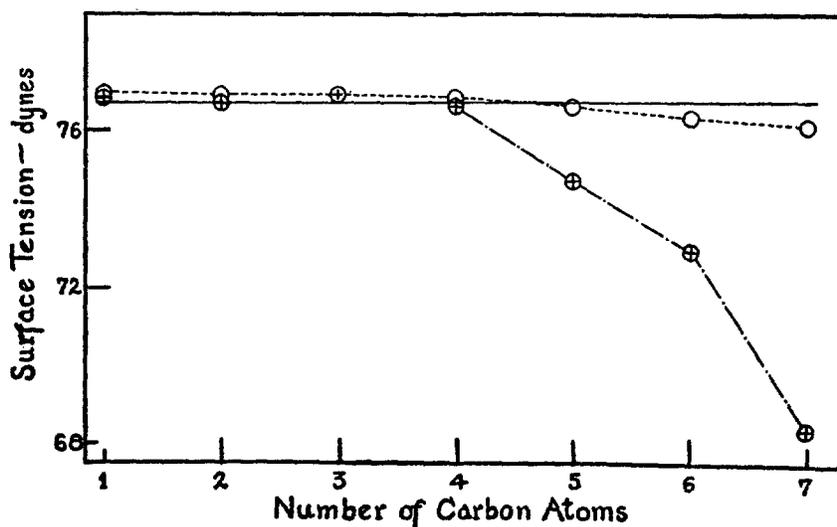


FIG. 3. Surface tension in dynes for each of the seven salt solutions plotted against the number of carbon atoms in the respective molecules. The solid line represents the surface tension of sea water; the dotted line connects the surface tension values for the equally effective concentrations along the continuous line of Fig. 2; the dashed line represents the surface tensions of the 0.028 molar solutions. Temperature, $17.2 \pm 0.2^\circ\text{C}$.

concentrations of the seven salt solutions, and of each salt solution at the same concentration (0.028 M). These data are represented in Fig. 3, showing that for the first four members of the series the surface tension is constant and only very slightly greater than that of sea water. For the equally stimulating concentrations the surface tensions of the higher members tend to decrease very slightly, but for the same concentrations (0.028 M) throughout the series, the surface

tension decreases noticeably beginning with valerate. The significance of these values will appear in the following discussion.

DISCUSSION

It is assumed that the reaction measured is a function of the intensity of stimulation of the receptor surface; that it is reversible, and that it may be quantitatively correlated with the available energy of the particles initiating the series of events called stimulation in the heterogeneous system of environment and receptor. Among the normal primary aliphatic alcohols previously studied the potential of the polar group was considered constant throughout the series and was held to function primarily as an orienting group. The stimulating efficiency of successive members of the series was then correlated with the non-polar portion of the molecule and its power to initiate changes at the receptor interface (Cole and Allison, 1930-31). In the normal aliphatic acid series the potential of the polar group of propionic acid and all the higher members of the series is constant, roughly the same for acetic, but considerably different for formic (Langmuir, 1929). That this higher potential of the formic polar group must be considered in stimulation of some receptors has been demonstrated for the earthworm by Crozier (1918), and for the sunfish by Allison (1931-32). To test the stimulating efficiency of the charged anion resulting from the dissociation of the aliphatic acids, it is necessary to use their salts, thereby eliminating any effect of the hydrogen ion. Equal degrees of dissociation for each salt may be assumed. The greater attraction of the polar group of the salts for water reduces the tendency of those salts to concentrate at an air-water or an air-oil interface. In fact, at an air-water interface the first few members of the series tend to increase the surface tension slightly when used over a certain range of concentrations. As the length of the carbon chain increases, however, the tendency to concentrate at the interface increases until lowering of the surface tension is the predominant effect (*cf.* Fig. 3). At an air-oil interface lowering of the surface tension is the predominant effect from acetic acid up, but the differences between the lower members are slight in dilute solutions (Donnan and Potts, 1910). If stimulation by the sodium salts of the fatty acids were directly correlated with the tendency of the anion to concentrate or to orient at the

receptor surface, and if the polar group acted primarily as an orienting group, then little difference would be expected between the effectiveness of the first members of the series. As the length of the carbon chain increased the tendency of the anion to orient at the interface would become dominant, and stimulating efficiency would be correlated with the length of the carbon chain. If the potential of the polar group were the only factor to be considered, then sodium formate would be more efficient than sodium acetate, but the higher members of the salt series would not differ much from the acetate, regardless of the length of the carbon chain. A relative measure of the potentials of the polar groups in a homologous series, such as the fatty acids, may be obtained by comparing the ionization constants of the members of the series (*cf.* Langmuir, 1929, above). The pK for formic acid is less than that for acetic acid. For acetic acid the pK is slightly less than for propionic acid, but for the next five acids used in the experiments the pK is practically constant. This means that sodium formate would hydrolyze less than acetate; the acetate slightly less than the propionate, but for the next five salts the amount of hydrolysis would be practically constant. In other words, in a series of equal concentrations there would be less free formic acid in the formate solution than free acetic acid in the acetate solution and a higher concentration of the formate anion than of the acetate anion. Similarly in the acetate solution there would be slightly less free acetic acid than free propionic acid in the propionate solution and a slightly higher concentration of the acetate anion than of the propionate anion. For the next five solutions the amount of free acids and the concentration of the anions would remain practically constant. The same sort of relationship has been demonstrated between the electron sharing ability of the organic radicals in the fatty acids and their ionization constants (Hixon and Johns, 1927).

In sea water solutions of the sodium salts of the fatty acids the dissociated anion of the salt is abnormal to a marine environment in the sense that it is usually absent. Even though the salt has no effect upon an air-water interface, or raises instead of lowers the surface tension because of the repulsion of the non-polar portion of the anion by water, there would be a definite tendency to replace other anions near the surface. If the receptor interface is altered by such

replacement, there would be some correlation between length of carbon chain and stimulating efficiency, since as the number of CH_2 groups increases the anion concentration at the interface would increase, thereby shifting the dynamic equilibrium at the interface. In stimulation of the barnacle by salts of the fatty acids, then, both the potential of the anion and the concentration of that ion near or at the receptor interface as determined by the length of the carbon chain, must be considered (*cf.* Cole and Allison, 1930–31). Sodium formate is more effective than sodium acetate because of the greater potential and mobility of the formate ion. Propionate is about as effective as acetate, being only slightly more effective than acetate, but less than formate. Beginning with butyrate the length of the carbon chain begins to play the predominant rôle, so that the stimulating efficiency of the higher members progressively increases as the number of CH_2 groups increases. The maximum effect is reached, however, in caproate, since heptylate shows no increased effect. If the formate is used in high enough concentration (*i.e.*, to produce 60 per cent closure), then it is less effective than acetate, a result correlated with the length of the carbon chain, and the tendency of the anion to concentrate at the interface.

SUMMARY

1. Stimulation in the rock barnacle *Balanus balanoides* by the sodium salts of the first seven normal aliphatic acids has been studied at several different concentrations for each salt. The pH was adjusted to that of sea water (8.1 ± 0.15) and all experimental conditions were held as constant as possible. Criterion of response was the per cent closure of valves at successive 2 minute intervals.

2. In general, the stimulating efficiency increases with concentration, but the ratios of effectiveness at increasing concentrations differ for each salt.

3. The order of effectiveness for 40 to 50 per cent closure is: heptylate = caproate > valerate > butyrate > formate > propionate > acetate. For 60 per cent closure or more, formate is the least effective of all.

4. Stimulating efficiency is correlated with the potential of the anion of the acid and with the concentration of that ion near or at the receptor surface as determined by the length of the carbon chain.

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