animals for testing chemical stimulation by substances added to sea water. Previous experiments have indicated that at such times there must be already present in the sea water some naturally occurring substances which stimulate the animals to become irregular or to close. It was suspected that one cause might be volatile oils set free by decomposing dinoflagellates in the sea water. These animals at Mt. Desert Island usually occur in small numbers but occasionally during the summer their population suddenly increases enormously. During the summer of 1934 on 34 successive days of July and August the population of diatoms, dinoflagellates and a few other of the larger microscopic plants and animals were determined and correlated with the number of regularly active animals in two sets of barnacles totaling 189 individuals.

Using percent closure as a criterion of behavior, it was found that no correlation exists between behavior and the number of diatoms, or algae, or total suspended materials in sea water. A definite correlation, however, was indicated between percent closure and the number of dinoflagellates per liter of sea water. Whenever the population of these animals became unusually large a corresponding large percentage of closed barnacles was found. This result is interpreted to mean that some product of metabolism or of decomposition of dinoflagellates causes closure of the barnacle if present in sufficient quantity. Further experiments are planned to identify the responsible substances.

STIMULATION OF THE BARNACLE, *BALANUS BALANOIDES*, BY NaCl, Na_2SO_4, $NaNO_3$, GLUCOSE, GLYCEROL AND UREA*

WILLIAM H. COLE and JAMES B. ALLISON
Rutgers University

Further studies on the responses of the barnacle, *Balanus balanoides,* to chemical stimulation were made by testing the stimulating efficiencies of the three inorganic salts, NaCl, Na_2SO_4, $NaNO_3$, and of the three non-electrolytes, urea, glycerol and glucose. (Preliminary studies on the salts were done in 1932 and reported in the Bull. Mt. Desert Is. Biol. Lab., for 1933, p. 29).

The experimental procedure was identical to that described in previous reports, using a temperature of 17 $\pm$ 0.2°C., a rate of flow of 250 $\pm$ 25 cc. per minute, and a recovery period of 30 minutes between tests. Each of two populations of 75 and 114 animals respectively was tested by different observers for each solution. The average volume of the 2 containers was 625 cc. The number of animals showing normal and regular cirral movements was recorded at the beginning of each test and at each successive minute up to and including 10 minutes. From such figures the percentage of regularly active animals was calculated for each interval on the basis of those regularly active at the beginning. Since in 169 of the total of 174

pairs of tests closely similar percentages were found for each of the two populations the data were combined by averaging the results from the two populations. The 4-minute interval was selected for comparative purposes, so that all of the percentages mentioned in this report are for the end of 4 minutes. Tests were made on 13 concentrations of the salts from 0.03 to 0.15 N at intervals of 0.01 N, and on 13 concentrations of the non-electrolytes, glucose and urea, from 0.06 to 0.2 M at intervals of 0.01 M (except the last 3 concentrations: 0.16, 0.18 and 0.2 M). Nineteen concentrations of glycerol from 0.06 to 0.28 M were also tested. Except for glycerol Merck's Reagent chemicals were used. The tests with glycerol were made with two different samples of Merck's Reagent and Baker's C.P. Analyzed and gave consistently different results. It was concluded that impurities such as fatty acids and fatty acid esters were responsible for the differences in results, and no attempts were made to analyze the data. These tests will have to be repeated with more highly purified material. Twelve mixtures of the salts and non-electrolytes were also tested as described below.

As reported in 1933 each one of the salts and also the non-electrolytes produced 2 contrasting effects on the barnacle depending upon the concentration used. At the lower concentrations the number of regularly active animals increased, while at the higher concentrations the number decreased. This means that there are two concentrations for each substance which are non-stimulating. Between these two critical concentrations the animals are induced to open and to remain regularly active; above the higher of the two concentrations the animals close in a definite way according to concentration. This double effect may be analogous to the common accelerative and depressor effects of certain drugs.

When percent opening and closure are plotted against concentration similar curves are obtained for each substance. Between the two critical concentrations the curves for percent opening are roughly cup-shape, reaching a maximum about midway. Above the higher critical concentration the curves for percent closure are symmetrically S-shape. Insufficient data are available for quantitatively expressing the "opening" effect. For closure however the curves may be represented by the following general expression:

$$\% \text{ closure} = \frac{M\, e^{b(C-a)}}{1 + e^{b(C-a)}}$$

in which M is the maximum closure percentage obtained experimentally; e is the base of natural logarithms (2.71 . . .) ; b is a velocity constant indicating the rate at which M is reached, thus determining the steepness of the curve; and a is the concentration at which one-half of the maximum percentage is obtained. The curves are thus symmetrical about the value of a. The curves and the equations differ only slightly in the values of the various constants as shown in Table I.

TABLE I

Substance	*Higher critical concentration*	*M*	*b*	*a*
NaCl	0.051	100	70	0.09 N
Na_2SO_4	0.062 N	98	65	0.1 N
$NaNO_3$	0.063 N	97	65	0.101 N
glucose & urea	0.075 M	94	57	0.118 M

The data for urea and glucose were so similar that they were combined. From a consideration of the values in any of the four columns in Table I it is possible to arrange the substances in order of effectiveness for closure as follows:

$$NaCl > 1/2Na_2SO_4 > NaNO_3 > \text{urea} = \text{glucose}$$

For all of the substances at certain concentrations a recovery effect was noted sometime after the second minute of exposure to the solutions. That is, the percentage closure decreased after a time before becoming constant. This effect was marked for $NaNO_3$ for all concentrations except the two highest; it was marked for Na_2SO_4 only at the lowest concentrations and much less marked for NaCl, urea and glucose at the lowest concentrations. In computing the percentages no correction for recovery has been introduced since only in a few cases of $NaNO_3$ (0.08, 0.09, 0.1 and 0.11 N) would the figures be significantly changed thereby. It is interesting to note that the order of recovery for the salts is just the reverse of the order of closure.

Three mixtures of urea and NaCl, 2 mixtures of Na_2SO_4 and $NaNO_3$, 2 mixtures of glucose and urea, 2 mixtures of glucose and NaCl, 2 mixtures of urea and $NaNO_3$, and 1 mixture of $NaNO_3$ and NaCl were tested. The results of these tests demonstrated that each one of the substances acts additively on the same group of receptors by bringing to the receptors a definite amount of energy. For example, if the higher critical concentration of NaCl is added to sea water plus 0.02 M glucose the effect of the mixture on the barnacle is the same as that produced by a solution of glucose alone equivalent to the higher critical concentration of glucose (0.075 M) plus 0.02 M glucose, or 0.095 M glucose.

The non-electrolytes, glucose and urea, were tested with the inorganic salts to determine whether or not closure is correlated with the change in the number of particles in solution, or with change in osmotic pressure. Since the two substances so dissimilar chemically are equally effective as stimulating agents it might be suspected without further data that osmotic pressure changes were responsible for the responses. However, the changes in osmotic pressure produced by these substances are just about one-half those produced by equally effective concentrations of the salts. It is therefore impossible to correlate effectiveness of all five substances with changes in osmotic pressure. The effect of the sodium salts can primarily be correlated with the equivalent concentration of the sodium ion. The slight dif-

ferences in effect of the three salts can be attributed to the differences in the structure and polarity of the three anions associated with the sodium ion. Stimulation by urea and glucose can be primarily correlated with their molar concentrations, which are almost equal in effect to corresponding normal concentrations of the sodium ion. In other words an uncharged particle such as glucose or urea disturbs the equilibrium between receptor and environment almost to the same degree as a charged particle such as sodium ion or any of the three associated anions. Although the energy changes at the receptor surface may be the same quantitatively in both cases, the mechanism by which the changes are produced may be entirely different. A complete explanation of such energy changes must await a more thorough knowledge of the physical chemistry of the established equilibrium existing between the chemo-receptors and their sea water environment.

* Part of the expenses of this investigation was defrayed by a grant-in-aid of the National Research Council.