and diminution in total volume of the animal. The maximum body cavity presure registered by *Cucumaria* ranged from 14.7 m.m. Hg by an animal 13 c.m. long, to 88.7 m.m. Hg by an animal 28 c.m. long when fully extended.

The pressure was measured as follows: To a mercury manometer was attached a Y tube. To one arm of the Y was attached a device connected with a rubber balloon and capable of being fastened to the body wall by a tight connection. The animal was anesthetized in magnesium sulfate solution, and the balloon inserted deflated through the body wall and fastened in place. The balloon was then slightly inflated through the other arm of the Y and its pressure measured. On transferring the animal to fresh running sea water recovery from the anesthetic was rapid. Mechanical stimulation by pricking or scratching the surface, handling, etc., brought on the usual contraction response. The rapid increase in internal pressure was registered by the manometer. The maximum increase in internal pressure found was approximately 16% above atmospheric pressure. This can be accounted for by the action of the body wall muscles alone.

STIMULATION BY ACIDS AND SALTS IN THE BARNACLE, *BALANUS*, AND THE KILLIFISH, *FUNDULUS*

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Continuing the study begun four years ago, the stimulating efficiencies of various acids and salts were determined on the rock barnacle, *Balanus balanoides*, and the killifish, *Fundulus heteroclitus*.

The acid solutions were prepared by making up to eighteen liters with sea water the required amount of acid. The mixture was thoroughly shaken and left standing until the hydrogen ion concentration as measured by the quinhydrone electrode became relatively constant. An interval of from thirty minutes to an hour elapsed before the solution was used. Such a standardized procedure was necessary because the addition of an acid to sea water which has a bicarbonate buffering system results in the liberation of carbonic acid. The change in pH of the sea water is an approximate measure of the amount of carbonic acid produced when all of the acid added to the sea water has reacted to liberate the weak acid. Under these conditions equivalent concentrations of strong and weak acids change the pH of sea water to about the same degree. For example, 0.001 N HCl, formic and acetic acid solutions prepared as described changed the pH of sea water from 8.3 to 6.32, 6.40 and 6.40 respectively. When the same concentration of these acids were made up in car-

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bonate-free sea water adjusted to a pH of 8.3, the pH fell to 3.00, 3.45, and 3.90 respectively. In untreated sea water at 0.002 N and above the difference in the strengths of the acids became apparent, since at 0.0025 N, hydrochloric acid changed the pH to 3.68, formic to 4.17, and acetic to 5.00.

The salt solutions were prepared by dissolving the appropriate amount of salt in sea water and making up to ten liters. The mixture was thoroughly shaken and allowed to stand for fifteen minutes before using. The pH of the solutions as determined colorimetrically did not change significantly from the pH of sea water.

The experimental procedure was similar to that described previously. The animals were placed in small dishes through which was passed running sea-water or experimental solution at 250 cc. ± 15 cc. per minute at 17.0 ± 0.3° C. For the barnacles the tests consisted of turning off the sea-water and turning on the experimental solution and recording the number of animals regularly active at one minute intervals until the number became constant. The solution was then turned off, the animals thoroughly rinsed with excess sea water and allowed ample time for recovery with the flow at 250 cc. per minute. The percent closure at two minute intervals was calculated and used as the criterion of response. For the killifish the time between the turning on of the solution and the first visible response of the opercula was recorded with a stop watch. The fish was then rinsed and allowed time for recovery. Easily reproducible results for both animals indicated that all toxic and adaptation effects were absent.

The experiments fell into three groups and are described accordingly.

I.

STIMULATION BY MINERAL ACIDS AND BY THE NORMAL ALIPHATIC ACIDS AT DIFFERENT HYDROGEN ION CONCENTRATIONS IN THE BARNACLE.

A tripartite population of 222 animals was used. The results for the three groups were almost identical, and were averaged. The following acids were tested at from 8 to 14 concentrations each; ranging from 0.0002 to 0.002 molar: formic, acetic, propionic, butyric, valeric, caproic, heptylic, hydrochloric, sulphuric and nitric. The hydrogen ion concentration varied from 0.32×10^{-7} to 58.89×10^{-7}. The four-minute interval was selected as the most advantageous for comparing all the acids. Percent closure at the end of four minutes was therefore plotted against hydrogen ion concentration, resulting in a family of exponential curves with increasing slopes from the formic and mineral acid curve to the heptylic curve. All of the curves coincide at the lowest hydrogen ion concentration and all become asymp-

totic near 100% closure but at different hydrogen ion concentrations. In other words, at low concentrations of the hydrogen ion all ten acids are equally effective, but as the hydrogen ion concentration increases, the higher fatty acids (beginning with butyric) become increasingly more effective as stimulating agents. Stimulation by acids must be related to the polar nature of the molecule combined with the field of force around the non-polar portion of the molecule, if present. For the mineral acids the latter force is absent so that stimulation may be considered as due solely to the polar nature of the molecule. For the first three aliphatic acids also the apparent stimulating force is related to the polar group, due to the fact that the carbon chain is so short and exerts a negligible effect. With butyric acid in the medium range of pH stimulating efficiency slightly increases; with valeric acid the increased efficiency appears at a much higher pH, and with caproic and heptylic acids still higher. Maximum closure with heptylic was obtained at $(H^+) = 8.5 \times 10^{-7}$, while for butyric it was 27×10^{-7}, and for the mineral acids, formic and acetic, it was about 35×10^{-7}.

Compared with the stimulating efficiencies of the fatty acids salts as determined last year at constant pH, it appears that the barnacle is about 10 times more sensitive to the changes produced in the environment by the free fatty acid than by the corresponding sodium salts.

II.

STIMULATION BY SODIUM AND POTASSIUM SALTS IN THE BARNACLE

Using the same technique as described above the stimulating efficiencies of the following salts were tested on the barnacle: sodium chloride, sodium nitrate, sodium sulphate from 0.02 to 0.15 N; potassium chloride, potassium nitrate and potassium sulphate from 0.0006 to 0.032 N using from 9 to 14 different concentrations each. All of the higher concentrations induce closure, and the percent closure decreases with concentration according to a complex relationship, not yet analysed. At the lower concentrations the opposite effect, or opening of the valves, is produced and the per cent opening increases to a maximum and then decreases as the concentration decreases. Two types of stimulating effect are thus revealed, analogous perhaps to the familiar exciting and depressor effects of certain drugs and narcotics. The order of effectiveness expressed by normal concentration is as follows: $KNO_3 > KCl > K_2SO_4 > NaCl > Na_2SO_4 > NaNO_3$. Potassium salts are on the average about five times more effective than sodium salts. Among the sodium salts the order of anion effectiveness is $Cl > SO_4 > NO_3$; while among the potassium salts it is: $NO_3 > Cl > SO_4$. A satisfactory interpretation and analysis of these results must await further experimentation.

III.

STIMULATION OF *Fundulus* BY HYDROCHLORIC AND BY FATTY ACIDS AT DIFFERENT HYDROGEN ION CONCENTRATIONS IN SEA WATER

The stimulating efficiencies of hydrochloric and the first seven aliphatic acids were measured on *Fundulus* by recording the reaction time of the fish at from five to seven different concentrations for each acid. The pH of the solutions varied from 3.1 to 7.0. By plotting reaction time against hydrogen ion concentration a curvilinear relationship was revealed for each acid. Hydrochloric and formic acids were about equal in stimulating effect, but beginning with acetic acid there was a progressive increase in stimulating efficiency with increase in the length of the carbon chain, except that heptylic acid was intermediate in effect between caproic and valeric acids. To give a reaction time of 10 seconds the following hydrogen ion concentrations were necessary for each acid; caproic, 0.05×10^{-7}; heptylic, 0.34×10^{-7}; valeric, 0.43×10^{-7}; butyric, 0.81×10^{-7}; propionic, 0.92×10^{-7}; acetic, 1.05×10^{-7}; formic and hydrochloric, 1.18×10^{-7}.

Compared with the barnacle *Fundulus* is much less susceptible to the disturbances in the environment produced by increasing the hydrogen ion concentration, and the progressive increase in stimulating effectiveness with increase in the number of $-CH_2$ groups begins lower in the series and is more pronounced.

STIMULATION BY THE DI-CARBOXYLIC ACIDS IN THE BARNACLE AND THE KILLIFISH

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The stimulating efficiency of the first four normal aliphatic dicarboxylic acids was investigated on *Balanus balanoides* and *Fundulus heteroclitus*. The acid solutions were made in sea water and allowed to stand in contact with air until equilibrium was practically attained. Thus no appreciable change in pH due to the liberation of CO_2 was observed while the solution was being used. The hydrogen ion concentration was measured by the quinhydrone electrode. The acid was admitted into the reaction chamber at a constant rate of flow, (250 cc. per minute for the barnacle, 100 cc. per minute for the killifish) and at a constant temperature ($17^{\circ} \pm 0.3$°C.). Following each test the animals were thoroughly rinsed by excess sea water, and were allowed ample time for recovery before the next test. Recovery occurred in two minutes, and, since results could be duplicated, there was clearly no permanent effect of stimulation on the animals.

The solutions used on the barnacle varied from 0.0022 to 0.0002 N, with a pH range of from 4.8 to 7.6. The progress of stimulation was