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

followed by determining the percent closure of the barnacles at one minute intervals. When percent closure by all four acids at the end of four minutes is plotted against pH a sigmoid relationship is obtained. Such a result would be predicted on theoretical grounds. All four acids, when solutions of equal pH are compared, have the same stimulating efficiency, and no effect due to the increasing length of the carbon chain was demonstrated. The stimulating agent in the barnacle is either the hydrogen ion itself or some factor related to the hydrogen ion concentration, such as the CO_2 tension in the acidified sea water. In any case there is some change brought about at the interface between animal and solution initiating the chain of events resulting in a nerve impulse and the response of closure. This interfacial change must be due to a disturbance of equilibrium of some system at the receptive surface which results in stimulation. A change in the free energy at the interface must follow. One possible expression of this free energy change might be a difference in potential between the receptive surface and the surrounding solution. The E.M.F. set up would cause an electron flow which might initiate the catenary series of events called stimulation.

The solutions used on *Fundulus* varied from 0.005 to 0.001 N, with a pH range of from 3.0 to 6.3. The criterion of response was a marked change in gill rate, usually a complete cessation of gill movement or "gulping." The reaction time was measured by a stop-watch. In the killifish stimulation by the di-carboxylic acids is determined not only by a factor related to the hydrogen ion concentration, but also by some factor related to the length of the carbon chain of the acid. When solutions of the first four di-carboxylic acids of the same pH are compared, it is seen that stimulating efficiency increases with the length of the carbon chain. Four reaction times were obtained on each of five fish making a total of twenty readings for each concentration used. The study on malonic acid was repeated, using one fish for twenty readings instead of five fish. Essentially the same results were obtained with one fish as with five.

The variability of response, as measured by the probable error, decreases with reaction time, or increases as the hydrogen ion concentration decreases. At lower concentrations of the acids a factor related to the hydrogen ion concentration seems to be the predominant stimulating agent, while at higher concentrations some factor related to the length of the carbon chain of the acid is the most important agent. Thus, when solutions of the four acids at low concentration and at the same pH are compared, the differences in stimulating effect are slight, while at higher concentrations at the same pH the differences in stimulating effect are marked.

In both the barnacle and the killifish stimulation is determined by two factors, one related to the polar portion of the molecule as measured by the hydrogen ion concentration, the other related to the non-polar portion as measured by the number of CH_2 groups. In the

barnacle the sensitivity to factors related to the hydrogen ion concentration is so great that its sensitivity to changes in the carbon chain is masked. In the killifish, however, the effect due to factors related to the hydrogen ion concentration does not predominate over the effect related to the length of the carbon chain and stimulation is a resultant of factors related to both polar and non-polar portions of the molecule. Thus the barnacle is apparently more sensitive to changes related to hydrogen ion concentration than is the killifish, while the latter is apparently more sensitive to changes related to the length of the carbon chain. This difference in sensitivity of the two animals is correlated with differences in the constitution and function of the receptive surfaces.

SEROLOGICAL RELATIONSHIPS OF THE MOLLUSCA

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A study of the serological relationships of the Mollusca by means of the precipitin test, was begun recently at Rutgers University. The first essentials in this work are proteins typical of the species whose interrelationships are to be studied. These proteins are obtained by extracting the whole bodies of the animals to be studied. During the summer of 1932, extracts of *Chrysodomus decemcostata, Mya arenaria* and *Venus mercenaria* were made at the Mt. Desert Island Biological Laboratory.

Methods employed in making these extracts were as follows: whole bodies were ground in a mortar when the animals were small or by using a meat-grinder and mortar when the animals were large. The extracts were made in 0.9% saline when body fluids were scarce or in body fluids themselves when they were of sufficient volume to make at least 100 c.c. of extract. All animals were starved before grinding. They were filtered through ordinary filter paper to remove large particles of flesh, and afterwards through a Seitz bacteriological filter using one asbestos pad. The fluid was thus cleared, and it was easier to pass through a second filtration. If more than 100 c.c. was present, it was reduced to the desired volume by evaporation (hastened by an electric fan). The extract was kept on ice at all possible times to prevent decomposition. Then it was refiltered through a sterile Seitz filter using two asbestos pads. The extract came through sterile as tested by allowing it to stand for 48 hours at room temperature after it had been bottled in sterile 5 c.c. vials. The filter, all instruments, pipettes, vials and rubber stoppers were sterilized by being subjected to a steam pressure of 15 pounds for one and a half hours. The extract was bottled immediately following filtration to insure against contamination.

These extracts, along with others, will be injected into rabbits to