change in the salt concentration of the environment and there is no apparent break in the relationship stated by equation (2) over the range of concentrations tested.

STIMULATION BY THE DICARBOXYLIC ACIDS AND THEIR DERIVATIVES IN *FUNDULUS HETEROCLITUS*

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The stimulating efficiency of the first six dicarboxylic acids was investigated on *Fundulus heteroclitus* in both salt and fresh water media. Eleven hydroxy or double bond derivatives of these acids were also studied in salt water only. The experimental procedure was the same as that described last year (BULL. MT. DESERT IS. BIOL. LAB., 1933, pp. 30-32). The temperature was 18°C.±0.3°, and the pH of the experimental solution varied from 2.7 to 7.0 inclusive. Since it was known from previous studies that tests on single individuals are as reliable as the averages from several animals, only one fish was used for all the experiments with salt water solutions. The number of observations was the same for each solution, so that the percentage probable error of the mean could be used as a measure of the variability of response. In view of the fact that every animal is limited to a maximum rate of response, determined by the neuro-muscular mechanism, the actual reaction time in seconds should be corrected by subtracting the minimum time in which the animal can respond under the given experimental conditions. For these experiments four seconds is subtracted. Four thousand three hundred observations were made on 215 different concentrations of the 18 acids.

The data obtained indicate that a parabolic relationship exists between the rate of reaction and the H^+ concentration for the dicarboxylic acids and their derivatives when *Fundulus* is stimulated by these acids in salt or fresh water. This means that the course of receptive processes which leads to stimulation is the same in both media, but differs quantitatively. Stimulation of *Fundulus* by these acids may be expressed by the following equation:

$$(\text{rate of reaction}) = K\ (H^+)^n$$

where K and n are constants.

Expressing this in terms of the corrected reaction time and the actual H^+ concentration the equation becomes:

$$\frac{100}{\text{R.T.} - 4} = K\ (H^+ \times 10^7)^n$$

The constants in the equation are characteristic of the particular acid, the fish, and the environment.

It may be pointed out that if the corrected reaction time instead of its reciprocal had been considered then a hyperbolic relationship between $(\text{R.T.} - 4)$ and $(H^+ \times 10^7)$ would appear of the type:

$$\text{R.T.} - 4 = \frac{100}{\text{K}\,(\text{H}^+ \times 10^7)^n}$$

Obviously such treatment would not alter the interpretation.

An understanding of the empirical constants in the equations would, of course, lead to a better comprehension of the various factors involved in stimulation. The constant n shows how rapidly the rate of response changes with H^+ concentration. It might be considered as a measure of the efficiency of the mechanism which relates reaction time to the (H^+) of a given acid and it is characteristic of the receptive process under the experimental conditions used.

If it is assumed that the amount of energy change at the receptor interface is directly related to the rate of response, then an acid with a high n value will bring about this energy change more rapidly than an acid with a low n value. While the course of reaction is the same in salt and fresh water, the receptive processes, the efficiency or velocity of which is measured by n, are characterized by a more efficient catenary series of events in fresh water than in salt. This difference is reasonable, for the effect of fresh water upon the orrientation of acid molecules at an interface might be quite different from the effect of salt water upon such a system. For a given environment the dicarboxylic acids all have the same value for n, i.e., the change in rate of reaction produced by a given change in equilibrium of the system as measured by the H^+ concentration is the same for all those acids. It is not surprising that the mechanism of the reaction or the relationship between rate of reaction and H^+ ion concentration should be the same for all the dicarboxylic acids, since they all have the general formula $R(COOH)_2$, where R represents the number of CH_2 groups.

The other constant, *K* in the equations is the value of 100 times the reciprocal of the reaction time when $(H^+) = 1 \times 10^{-7}N$. It might be considered a relative measure of the amount of energy brought to the semi-permeable membrane by a given acid. The dicarboxylic acids differ only in the number of CH_2 groups present. Since the introduction of each CH_2 group into a molecule results in the addition of a definite increment of energy to that molecule, a corresponding increase of the constant *K* in the equation for stimulation could be expected. This is actually the case, for *K* increases exponentially for the dicarboxylic acids where the exponent is a function of the number of CH_2 groups. This holds true up to succinic acid. Beyond this acid a further increase in the number of CH_2 groups brings about no further change in the energy relationship at the receptor interface. *K* is characteristic of the acid in relationship to its environment. Thus the *K* value for an acid is different in fresh water from that in salt. The rate of change of *K* as the number of CH_2 groups increases in the acid series is also a function of the environment and of the experimental solution. The value of the rate of reaction at $(H^+) = 1 \times 10^{-7}N$ is dependent on the fish as well as on the acid for while all killifish will give the same constant, n, the values for *K* will be distributed about a mean.

Since *K* is considered a measure of the available energy of an acid molecule which plays a role in the receptive processes, a relationship between *K* and other measures of the free energy of the molecule might be expected. Such a relationship would be apparent only if both energy measures were similar functions of the total energy of the molecule. For example, heat of combustion increases by 153 Cal. for each CH_2 group which is introduced into the dicarboxylic acid molecule. *K* increases by approximately 2.9 units up to succinic, beyond which there is little change with increasing CH_2 groups. The dissociation constants rapidly decrease up to succinic, beyond which there is little change. Thus a relationship may exist between *K* and the physical and chemical constants in so far as these particular constants may be a relative measure of the free energy of a molecule which is involved in stimulation.

A study of the dicarboxylic acid derivatives was chiefly a comparison of certain sterioisomers. Those used as stimulating agents were maleic and fumaric acids, d, l, and i malic acids, d, l, i, and m tartaric acids, saccharic, and mucic acids. In general it may be stated that those isomers having very similar physical and chemical properties have the same constants in the equation, while those of widely different properties have different constants in the equation. In general the more chemically active member of an isomeric pair is the more efficient stimulating agent. To illustrate these points two examples will be considered. All the isomeric tartaric acids have very similar physical and chemical properties and they give the same constants in the equation for stimulation. Both maleic and fumaric acids have practically the same heats of combustion and give the same *K* value in the equation. They differ widely in other properties, maleic being the more active chemically. Maleic is also the more efficient stimulating agent as shown by its higher *n* value in the equation.

For the dicarboxylic acids the order of effectiveness in stimulation is: pimelic = adipic = glutaric = succinic > malonic > oxalic. For the derivatives of the dicarboxylic acids the order of effectiveness depends on the concentration.

STUDIES UPON RENAL FUNCTION IN MARINE TELEOSTS

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1. The effect of blockage of the gastro-intestinal tract upon urine formation in a marine teleost, *Myoxocephalus octodecimspinosus*. With DAVID ENNIS.

Obstruction of the gastro-intestinal tract of the sculpin at the pyloric end of the stomach results in a marked shut-down in urine formation, marked loss in body weight, and ultimately death. In the later stages the chloride content of the urine falls to low values or even to zero, while PO_4 continues to be excreted. Our experiments confirm the interpretation that the regulation of body weight and blood composition in marine teleosts is effected primarily by the con-