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HYDROGEN-ION CONCENTRATION AND THE RHYTHMIC ACTIVITY OF THE NERVE CELLS IN THE GANGLION OF THE LIMULUS HEART

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The influence of the hydrogen-ion concentration on the rhythmic activity of the nerve cells in the respiratory center of the vertebrates has been much discussed (Gesell, 1925). Another form of rhythmic activity of nerve cells is represented by the regular nervous impulse originating in the ganglion of the *Limulus* heart, and the influence of hydrogen-ion concentration on these nerve cells can be studied with great convenience. In 1906 Carlson made a preliminary study of the action of acids and alkalies on the ganglionic rhythm. He observed that addition of 1 part of a 0.6 N NaOH or KOH to 100 parts of sea water or plasma had a stimulating effect on the rate and strength of the nervous discharge from the ganglion. A main factor in this effect, however, appears to be the removal of the Mg-ion as $Mg(OH)_2$, the precipitation of which is readily seen in such an alkaline medium. Carlson also pointed out the difference between the action of strong bases like KOH and NaOH and weak bases like NH_4OH. No such difference, however, was observed with strong and weak acids, including HCl, H_2SO_4, oxalic, citric, acetic, tartaric, malic and formic acids. All these acids produce a primary increase in amplitude and frequency of the heart beat, followed by cessation of the rhythm (Carlson, 1906). Carbonic acid apparently acts in a different way, for Newman (1906) observed a primary increase in the amplitude of the heart beat, associated with a marked decrease in frequency, when CO_2 was bubbled through the sea water surrounding the ganglion. More recently, Asher and Garrey (1930) have repeated Newman's work; they found that CO_2 often had little effect on the ganglion for some time, then quite suddenly the contractions became seriously impaired. No particular attention was paid to the influence of hydrogen-ion concentration as such in these earlier experiments. Variation of either the undissociated acid or the hydrogen-ion concentration in the medium (or of both) might conceivably be factors in the quantitative differences just referred to. Accordingly it seemed desirable to repeat some of these observations and to study further the relation of the hydrogen-ion concentration to the rhythmic activity of the ganglion in solutions of strong and weak acids and bases.

The experiments to be described were all carried out on the ganglion of the *Limulus* heart. The ganglion was isolated posteriorly, while remaining in connection anteriorly with two segments of the heart musculature. The muscle was kept immersed in sea water and the contractions were recorded graphically. Test solutions were applied to the ganglion alone, and the hydrogen-ion concentrations were determined colorimetrically.

In the study of the influence of the hydrogen-ion concentration, two kinds of effects should be distinguished: (1) the action of the hydrogen-ion in the medium as such, and (2) the secondary effect of other ions or molecules introduced with the acid or base. For the simple effect of the hydrogen-ion concentration as such, both strong mineral acid and base (e.g., HCl and NaOH) and buffer solutions were used. The effects of weak acid and base were also studied to bring out the difference between the penetrating and the non-penetrating substances.

THE ACTION OF ACIDS

The automatic rhythm of the ganglion is not very responsive to changes of hydrogen-ion concentration as such. Addition of HCl to the unbuffered Ringer's solution[1] (Chao, 1933) produces no well-marked change on the ganglion until the acid reaches a more or less toxic concentration of about N/1,000 HCl. At this concentration the rate of the nervous discharge is accelerated considerably; the intensity of the discharge (as measured by amplitude of contraction) may be slightly increased at first but rapidly diminishes; and the rhythm becomes irregular, showing definite symptoms of injury. Experiments with Ringer's solution buffered with primary and secondary Na-phosphate (3 millimols PO_4 per liter) at pH 5.4 also showed no marked effect.

When CO_2 is bubbled through the Ringer's solution or sea water, the reaction of the solution becomes distinctly acid, but the effect on the ganglion is entirely different from that produced by HCl. As described by Newman (1906), the amplitude of the heart-beat may slightly increase at first; later it decreases with decreasing frequency of the nervous discharge. The degree of these changes appears to depend more upon the concentration of the undissociated carbonic acid molecules than upon the acid reaction. Thus, the average rate of the nervous discharge in 10 minutes for two experiments is -14 per cent

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[1] The unbuffered Ringer's solution, containing 445 millimols NaCl, 8.9 millimols KCl, and 37 millimols $CaCl_2$ in a liter, is slightly alkaline in reaction like the sea water (pH about 8.2), due to traces of $Ca(OH)_2$ in the $CaCl_2$.

at pH 6.2 and −38 per cent at pH 5.7 as compared with the normal rate in sea water (pH 8.2). At pH 5.2 (at which sea water is practically saturated with CO_2) the rhythmic activity is inhibited in about 5 minutes (average of 7 experiments). The same pH (5.2) can be obtained by addition of about 2 millimols HCl to a liter of sea water, but this solution produces a slight increase in rate; the average rate in 10 minutes for two experiments is increased by 10 per cent as compared with the normal rate in sea water.[2] When the pH is decreased to 4.0 by addition of more HCl, the average rate in 10 minutes is increased by 17 per cent (average of 4 experiments). Further addition of HCl (3 millimols per liter sea water) may increase the rate 50 per cent or more, often followed by serious impairment of the rhythmicity. These experiments indicate clearly that the external hydrogen-ion concentration is not the determining factor in the action of the CO_2-saturated solution. The action of this medium must apparently be attributed to the undissociated carbonic acid molecules which are known to penetrate living cells with great readiness.

Acetate buffer (10 millimols acetate per liter) dissolved in sea water or Ringer's solution produces the same general effect but appears to be less effective than the CO_2-saturated solutions at the same pH. Thus at pH 5.2, the CO_2-saturated sea water inhibits the rhythm in about 5 minutes on the average, but the acetate-buffered sea water produces only a decrease of 24 per cent of the average rate in 10 minutes (average of two experiments).[3] The concentrations of acetic acid and carbonic acid in the acetate-buffered and CO_2-saturated sea water respectively are, however, not the same; that of carbonic acid being much the higher. The difference in the concentration of the undissociated acid molecules may account for this quantitative difference in action. Further experiments were performed in which different dilutions of acetate buffer were used at approximately the same pH; these showed clearly that the effect on the rhythm increases with increase in the concentration of acetate buffer.

The Action of Alkalies

Experiments with alkali in sea water can be performed within a rather narrow range of pH only, for Mg, which is present in high concentration (up to 53 millimols per liter) is precipitated at $Mg(OH)_2$ near pH 10. Many calcium salts (e.g., carbonate and phosphate) are also very slightly soluble in alkaline solution. Most of the experiments

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[2] Lactic acid acts like HCl in causing a primary increase in rate followed by decline and irregularity of beat, but is less effective.

[3] Acetate-buffered sea water can also inhibit the ganglionic rhythm but at a lower pH; the rhythm is inhibited in about 3 minutes at pH 4.4 (average of 5 experiments).

on the action of alkalies were performed by adding the alkali to the simple Ringer's solution which does not contain any Mg-ion.

The pure effect of OH-ions as such can be demonstrated by adding NaOH to the Ringer's solution. No well-marked effect is seen until the concentration of NaOH reaches N/1,000 or more.[4] In a Ringer's solution containing N/1,000 NaOH, there is either no change in frequency and amplitude or a slight decrease in frequency with a slight increase in amplitude. As alkalinity is still further increased, the decrease in frequency becomes more pronounced, the rhythm becomes irregular, and definite symptoms of injury appear.

Similar experiments were performed with NH_4OH to furnish a comparison between strong and weak alkalies. As described by Carlson (1906), addition of 1 part of a 0.6 N NaOH solution to 4,000 parts of sea water or plasma produces a rapid decrease in the intensity of the nervous discharge with a slight acceleration of rate. The same effect is obtained with NH_4OH in the Ringer's solution; with a somewhat higher concentration of NH_4OH (1 millimol per liter) the rhythmic activity is rapidly inhibited, often to complete cessation of rhythm. This inhibition is temporary. If the ganglion is allowed to remain in the solution containing NH_4OH, a gradual recovery follows; both the rate and the amplitude begin to increase and may finally attain a level well above the initial condition. The temporary inhibitory action of NH_4OH resembles the paradox phenomenon shown under certain conditions in the ganglion (Chao, 1934). The effect, however, is not due to the OH-ion, for it cannot be produced simply by adding sufficient NaOH to inhibit the rhythm completely. Nor can it be referred to the action of the NH_4-ion, for NH_4Cl has an entirely different effect on the ganglion. The addition of 5 millimols NH_4Cl in a liter of Ringer's solution or sea water causes a definite increase in the frequency of the nervous discharge[5] (*cf.* also Carlson, 1906). Apparently the peculiar behavior of NH_4OH is to be attributed to the specific action of the undissociated molecules of NH_4OH. These penetrate living cells readily, in contrast to the lack of penetration or slow penetration of strong alkalies in dilute solution.

In general, the results described in these experiments bring out

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[4] Ringer's solution buffered with boric acid and NaOH (2.5 millimols borate per liter) at pH 9.4 has no marked effect on the ganglion.

[5] NH_4Cl is slightly hydrolyzed so as to make the solution more acid. However, the slight stimulating action of NH_4Cl is not an effect of the acid reaction, for the solution can be kept alkaline by addition of a small amount of NH_4OH and yet produces the same effect on the ganglion; e.g., the addition of 0.2 millimol NH_4OH together with 5 millimols NH_4Cl to a liter of Ringer's solution (pH 9.2) still increases the rate. The action of NH_4-ion is, in fact, more like that of K-ion (Chao, 1933).

clearly the difference between the respective influences of weak and strong acids and alkalies on the ganglionic rhythm. The characteristic effects of HCl and NaOH on the rhythm are to be referred to simple changes of pH in the external medium; while in the case of weak acids (like acetic and carbonic acids) or weak bases (like NH_4OH) not only are their physiological effects different from those of strong acid and strong base, but their mode of action indicates the presence of special factors, connected apparently with the penetration of the undissociated molecules into the cell interior.[6]

SUMMARY

The automatic rhythmic activity of the nerve cells in the ganglion of the *Limulus* heart is relatively resistant to changes in pH in the external medium as such. Experiments with strong acid and base indicate that no well-marked physiological effect is seen until toxic concentrations are approached. Thus H-ion (N/1,000 HCl) produces a rapid irregular rhythm with decreasing amplitude of contraction followed by cessation of heart-beat, while OH-ion (N/1,000 NaOH) has an inhibitory effect. Weak penetrating acids (e.g., carbonic and acetic acids) inhibit the rhythm with primary increase in intensity of the nervous discharge, while the action of NH_4OH is characterized by a period of temporary inhibition. The contrast is to be related to the characteristic difference in the readiness of penetration of the undissociated molecules and the ions into living cells.

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[6] For a general discussion and literature, see Lillie, 1927, pp. 720–722.