THE EFFECT OF HYDROGEN ION CONCENTRATION ON SWELLING OF CELLS.

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It has been repeatedly stated in the literature that mammalian cells increase in volume when placed in acid solutions. Conversely, it has been thought that the swelling of tissue cells observed in various pathological conditions is often the result of acid production (1). The process has been supposed to be similar to that producing volume changes in such dead systems as gelatin, when the H^+ concentration of the solution is altered.

Erythrocytes are known to swell in acid solutions. This volume change has been shown by Van Slyke and his associates (2) and by Warburg (3) to be dependent on the facts that the membrane of the erythrocyte is permeable to anions, but not to cations or hemoglobin, and that the amount of base in combination with hemoglobin is a function of the H^+ concentration; alteration in the latter produces change in osmotic pressure within the cell, in accordance with the Donnan equilibrium, and hence volume change of the cell.

The mammalian erythrocyte is, however, a highly specialized cell, in respect to function, as well as low metabolism, inability to divide, and absence of nucleus. It is, therefore, not permissible to assume that a mechanism for volume change exists in living nucleated cells such as is present in erythrocytes and dead systems. In fact recent work tends to show that living cells are relatively impermeable to both cations and anions as long as they remain uninjured.

We were, moreover, inclined to question whether swelling of living cells in acid solutions was a well established fact. In previous work it is not clear whether cells were alive or dead at the end of the experiment. But, as will be seen, it is precisely this question of the life or death of the cell that determines whether it will swell in acid solutions.

The problem was, therefore, to determine whether the volume of living cells depends on the pH of the solution, as is the case with erythrocytes. It was necessary to select a type of cell that can be readily measured, that is more comparable, both in structure and function, to tissue cells than is the case with mammalian erythrocytes, and finally a cell of which the viability can be definitely determined. Unfertilized *Arbacia* eggs meet these requirements. In common with tissue cells they possess a nucleus, and the ability to divide. Large numbers of comparable cells can be obtained from a single animal and examined in sea water, their natural environment, hence without injury, except such as is deliberately induced. Since they are spherical, changes in volume can readily be calculated from changes in diameter. At the end of the experiment, viability can be tested by attempting fertilization.

Method.

Solutions were prepared as follows: Sea water was neutralized with HCl and aerated overnight by means of a water pump. HCl or NaOH was then added to obtain the desired range of pH values, from 3.0 to 9.8, measured colorimetrically. Pyrex tubes of 100 cc. capacity were nearly filled with these solutions and stoppered. Eggs were obtained from a single animal for each experiment. They were washed twice in sea water, concentrated by slight centrifugation, washed again in the solution to be tested, and a few placed in each tube, at room temperature. The tubes were frequently agitated. In control tubes were placed identical solutions, eggs, and a few drops of an indicator solution. Changes of pH during an experiment were found to be generally insignificant.

At stated intervals, samples of eggs were removed from the tubes. The diameters of 10 eggs were measured with a 10 mm. objective and an ocular screw micrometer. With this system a magnification of 240 diameters was obtained. The eggs were then returned to ordinary sea water and a freshly diluted suspension of sperm added. The cells were examined for cleavage after about 2 hours.

In a second series of experiments washed CO_2 was bubbled through neutralized sea water, or aqueous NH_4OH added to obtain the desired pH range (4.2 to 9.8). In control tubes, changes in pH during experiments were usually slight.

TABLE I.

Effect of H Ion Concentration (HCl and NaOH) on Volume of Unfertilized Arbacia Eggs. Control = 75.7 ± 0.4 μ in Diameter.

pH.....	3.0	4.0	5.0	6.0	7.0	8.0	9.0	9.8
Time.								
min.								
4	75.2 ± 0.4 C.	75.4 ± 0.3 C.	75.3 ± 0.3 C.	74.9 ± 0.3 C.	75.4 ± 0.4 C.	73.9 ± 0.4 C.	75.5 ± 0.3 C.	75.0 ± 0.4 C.
16	75.9 ± 0.3 A.C.	76.3 ± 0.3 C.	75.2 ± 0.2 C.	75.0 ± 0.4 C.	76.3 ± 0.4 C.	75.3 ± 0.4 C.	75.0 ± 0.3 C.	75.3 ± 0.3 C.
64	76.5 ± 0.6 N.C.	76.2 ± 0.5 N.C.	74.1 ± 0.2 A.C.	74.9 ± 0.3 C.	75.5 ± 0.4 C.	74.6 ± 0.3 C.	74.9 ± 0.3 C.	74.6 ± 0.2 C.
256	83.4 ± 0.5 N.C.	78.8 ± 0.8 N.C.	75.8 ± 0.2 A.C.	74.7 ± 0.3 A.C.	75.4 ± 0.2 C.	75.5 ± 0.2 C.	75.3 ± 0.2 C.	75.8 ± 0.4 C.

Each figure represents the mean diameter in micra, plus or minus its probable error, of 10 cells. After measurement the cells were returned to ordinary sea water and sperm was added; C. = normal cleavage; A.C. = atypical cleavage; N.C. = no cleavage.

Results of Exposure to HCl and to NaOH.

In Table I are given the results of one of three similar experiments. It will be seen that changes in volume occurred only in the two most acid solutions, *i.e.* at pH 3.0 and 4.0, and in these only after long exposure to the acid. An interesting and significant relation is brought out between volume change and the ability of the egg to develop: loss of power to develop precedes volume change. Thus at pH 3.0,

TABLE II.

Effect of H Ion Concentration (CO_2 and NH_4OH) on Diameter of Unfertilized Arbacia Eggs.
Control = 75.7 ± 0.1 μ in Diameter.

pH.....	4.2	6.0	7.0	8.0	9.0	9.8
Time.						
min.						
4	76.0 ± 0.2 N.C.	75.4 ± 0.3 C.	75.4 ± 0.2 C.	77.3 ± 0.3 C.	76.0 ± 0.2 C.	76.2 ± 0.3 C.
16	75.0 ± 0.3 N.C.	75.7 ± 0.2 C.	75.9 ± 0.3 C.	74.4 ± 0.3 C.	75.9 ± 0.3 C.	75.7 ± 0.2 A.C.
64	75.8 ± 0.2 N.C.	75.5 ± 0.2 C.	75.0 ± 0.3 C.	75.6 ± 0.2 C.	76.3 ± 0.3 A.C.	76.4 ± 0.3 A.C.
256	76.9 ± 0.3 N.C.	74.9 ± 0.2 A.C.	74.9 ± 0.3 C.	75.5 ± 0.3 C.	75.5 ± 0.3 N.C.	75.8 ± 0.3 N.C.

Each figure represents the mean diameter in micra, plus or minus its probable error, of 10 cells. After measurement the cells were returned to ordinary sea water and sperm was added. C. = normal cleavage; A.C. = atypical cleavage; N.C. = no cleavage.

after 4 minutes exposure, there was no volume change and normal cleavage followed fertilization. After 16 minutes, there was no change in size, but the eggs subsequently divided atypically. After 64 minutes there was no change in size, but the eggs failed to divide at all. After 256 minutes the eggs showed marked swelling and no cleavage occurred.

At pH 4.0 the changes were less marked, but in the same direction.

The fact, therefore, stands out clearly that no swelling of the eggs

occurred in solutions of HCl until *after* the eggs had suffered serious injury: injury so severe that they were incapable of subsequent development. That the eggs at the time of swelling were not only injured, but actually dead, is altogether probable.

Results of Exposure to CO_2 and to NH_3.

The effects produced by these substances were similar to those obtained with HCl and with NaOH. In Table II are given the results of a typical experiment. It is seen that, though there was marked inhibition of subsequent cleavage, there was, with the concentrations of CO_2 and NH_3 used, no change in size as long as the cells remained uninjured.

DISCUSSION.

That the results obtained with *Arbacia* eggs cannot, without experimental evidence, be applied to all cells, is sufficiently evident. Bearing this fact in mind, our conclusions may be stated as follows:

Our experiments tend to show that cells other than erythrocytes do not swell in HCl, NaOH, CO_2, or NH_3 as long as they are alive. That this is true of HCl and NaOH is perhaps not surprising, since recent work appears to show that uninjured cells are relatively impermeable to all ions. But with CO_2 and NH_3 the case is quite different. Here we are dealing with substances known to penetrate cells with great readiness and to alter their hydrogen ion concentration (4). Under these circumstances both erythrocytes and dead proteins such as gelatin would undergo volume change, due to alteration in the amount of protein in combination with acid or base. Possibly no such combination occurs in the case of living protoplasm. However that may be, our experiments appear to show that the volume of living cells, in contrast to that of erythrocytes and dead proteins, is independent of H^+ concentration.

Finally the fact remains that our results differ from those of previous workers who found that cells swelled in acid solutions. Whether this discrepancy depends on differences in material or whether the cells observed by former workers were injured or dead, remains for the present an open question. In general, however, it seems to be true that if the internal reaction of a cell is made acid enough to cause swelling, the cell is seriously injured (Crozier (5)).

SUMMARY.

1. The effect of HCl, NaOH, CO_2, and NH_3 on the volume of unfertilized *Arbacia* eggs was tested over a wide range of pH values.

2. No swelling occurred, except in HCl solutions, and there not until after injury or death had occurred.

3. Whereas the volume of erythrocytes and of proteins such as gelatin is known to be dependent on the pH of the solution, such a relation does not exist in the case of living and uninjured cells, at least of the type tested.

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