XCVI. THE EFFECT OF BICARBONATE IONS ON THE SWELLING OF GELATIN.

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AMONG the inorganic anions occurring in the body, the chlorides and bicarbonates are, in point of quantity, the most important. It is known that these two anions may, to a large extent, replace each other in the body without causing any very profound physiological effects (other than the change in the acid-base balance), though certain other anions are not to the same extent interchangeable without pathological results. In various physico-chemical relationships the different anions are known to produce markedly different effects, as is shown by the persistence of the lyotropic or Hofmeister series in many phenomena, including various aspects of protein-water equilibria.

The effects of chloride and nitrate on water imbibition by gelatin at varying salt concentrations, temperatures, and p_H , have been studied by Jordan-Lloyd and Pleass [1927, 1928]. At low concentrations the effects of these univalent anions follow the valency theory of Loeb [1922], but at higher concentrations the effects diverge—which is interpreted as being evidence of a lyotropic difference.

In view of the physiological importance and variability of the HCO_3 ion concentration, it seemed of interest to study the effect of this ion on gelatin swelling in a manner parallel to part of the work of Jordan-Lloyd and Pleass on the Cl and NO_3 ions. The feasible range of p_H for studying the bicarbonate effect by this method is limited. Above p_H 8 the concentration of the carbonate ion ceases to be negligible. At low concentrations of bicarbonate the "Loeb effect" becomes predominant; and the bicarbonate concentration places a lower limit on the range of p_H obtainable by equilibration with CO_2 . However, the feasible p_H range approximates to the physiological p_H range.

The gelatin was prepared by the method described by Jordan-Lloyd and Pleass [1927]. Twelve sheets of Coignet gelatin were put in 3 l. of 0.2 M NaCl , the p_H being adjusted to 3 by HCl ; the mixture was gently stirred; after several hours HCl was added to restore the p_H to 3. This was repeated until equilibrium had been reached. The fluid was then removed, and 3 l. of fresh 0.2 M NaCl at p_H 3 were added. After standing overnight the fluid was decanted and the gelatin washed with distilled water until the chloride test (AgNO_3) showed only a faint trace. The gelatin was air-dried. It contained 19.6–19.9 % water. Samples, ashed, gave negative oxalate tests for calcium.

Pieces of the gelatin weighing from 0.05 to 0.09 g. were placed in Gooch crucibles in the test fluid (1 cc. fluid to 0.001 g. gelatin) in small wide-mouthed bottles (fitted with rubber stoppers and aeration tubes), in a water-bath at $15^\circ \pm 0.5^\circ$. This temperature was chosen to keep the gelatin in the insoluble "A" form [Smith, 1919]. The test fluid being NaHCO_3 solution of known molarity, washed CO_2 was bubbled through, either from a generator or from a large Douglas bag filled with a CO_2 -air mixture of known CO_2 content. Equilibration was tested by analysis (Haldane method) of the gas mixture issuing from the bubbling chain after passing through the solutions. All stoppers, rubber connections, etc., were sealed with paraffin. The bottles were kept in the bath for 3 days. The crucibles and gelatin were then removed, gently dried with filter paper, and promptly weighed. Swelling was expressed as the wet-swollen weight in percentage of the dry weight of the gelatin, and was plotted against p_{H} .

Tests were made at four intervals along the p_{H} ordinate of the curves. The p_{H} of the solutions was calculated by the Henderson-Hasselbalch equation from the amount of base and the CO_2 tension. In the higher strengths, approximate correction for the Debye-Huckel effect was made by the formula [Clark, 1928] $\log \gamma = \frac{0.5\sqrt{\mu}}{1 + 3.3 \times 10^7 a\sqrt{\mu}}$. For a the value 4.2×10^{-8} was used [Kolthoff, 1928]. This correction amounted, in the high concentrations, to 0.2 p_{H} . The salting-out term was ignored. The value of p_{K} for carbonic acid was taken as 6.54 at 15° (calculated from data given by Hastings and Sendroy [1925]). A series of determinations by the quinhydrone electrode indicated that the calculated p_{H} was accurate to 0.15, which, in view of the scatter of weight results and the curve shapes, was satisfactory.

A few series of parallel tests with NaCl - NaOH and NaNO_3 - NaOH were run for comparison and to connect the results with the data of Jordan-Lloyd and Pleass. A few tests were also made with acetate. The gelatin was placed as before in sealed bottles with the salt mixtures; the p_{H} was determined electrometrically at the end of the swelling period, when the gelatin was weighed.

The water-swelling of gelatin does not reach a true equilibrium. The wet weight is not an altogether accurate measure of the amount of "water of swelling." "Gelatin" is perhaps not a homogeneous entity. The section of the curves studied was necessarily short. As a consequence of these and other facts, the results obtained are rather widely scattered from the means. (The maximum scatter was 276 between extremes of triplicates, though as a rule the agreement was much better. The standard deviation from the mean was 52.) By strict statistical methods, it would be difficult to state with a high degree of accuracy the position and orientation of the curves obtained. They seem, however, to be consistent with each other and with previous work and capable of yielding information of some value (see Fig. 1).

As can be seen from a comparison of the extended curves for chloride and nitrate effects obtained by Jordan-Lloyd and Pleass, it is obviously unsafe to

make, from a study over such a limited range of p_H and concentration, and using gelatin swelling as a criterion, any generalisation as to the position of the bicarbonate ion in a lyotropic series. Certain facts, however, do seem to be of interest. The swelling-suppression effects are, in this range and within the rather large experimental error of the method, of the same order of magnitude as for the chlorides, and greater than for the nitrates (the difference being evident in the higher concentrations). The swelling of the gelatin in 0.5 *M* sodium acetate at p_H 7.3 was 1690 % (average of three tests), lower by about 130 than the corresponding point in the 0.5 *M* bicarbonate curve. The usual Hofmeister series is acetate-chloride-nitrate-bromide [Bancroft, 1921]. These results suggest that, at least under these limited conditions, the HCO_3 ion lies approximately with the Cl ion in the series. It also appears that variations in p_H cause rather less variation in the effect on protein swelling (in that the curves are flatter) than do corresponding variations in chloride. While no physical or chemical explanation can be offered, these facts are of interest in view of the predominance of the chloride and bicarbonate ions in the body, their relative interchangeability, and the relative lability of the body bicarbonate and CO_2 concentrations.

Fig. 1.

SUMMARY.

The effect of varying bicarbonate concentration and p_H on gelatin swelling was studied over a limited range and found to be of the same order of magnitude as the corresponding effect of the chloride ion.

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