The Swelling of Collagen in Alkaline Solutions

3. SWELLING IN SOLUTIONS OF BIVALENT BASES

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The swelling of collagen in solutions of a number of univalent bases has been described in previous communications (Bowes & Kenten, 1950*a, b*). In the present paper the work has been extended to cover the bivalent bases, calcium, strontium and barium hydroxide.

EXPERIMENTAL

The raw material and technique were the same as those used in Parts 1 and 2 (Bowes & Kenten, 1950*a, b*). Saturated solutions of $\text{Ca}(\text{OH})_2$, $\text{Sr}(\text{OH})_2$ and $\text{Ba}(\text{OH})_2$ were prepared by shaking excess of the solid hydroxide with boiled distilled water and allowing to settle.

RESULTS

Swelling in solutions of calcium, strontium and barium hydroxides

The swelling of collagen in solutions of calcium, strontium and barium hydroxides was considerably less than in sodium hydroxide solutions at all pH

Fig. 1. Swelling of collagen in solutions of $\text{Ca}(\text{OH})_2$, $\triangle-\triangle$; $\text{Sr}(\text{OH})_2$, $+--+$; $\text{Ba}(\text{OH})_2$, $\square-\square$. Curve $\circ-\circ$ for NaOH solutions included for comparison.

values, and more especially in the range 11.0–13.0 (Fig. 1). The curves were of the same general shape as the sodium hydroxide curve, showing a flattening

between pH 12.0 and 13.0 followed by a sharp increase in swelling at higher pH values. Appreciable swelling commenced only at about pH 11, i.e. approximately one pH unit higher than with sodium hydroxide. The amount of swelling decreased in the order barium, strontium, calcium hydroxide. Even in barium hydroxide solutions the swelling, in excess of that occurring in the isoelectric range, was barely half that in sodium hydroxide solutions, that in strontium hydroxide was rather less and that in calcium hydroxide was only about one-eighth of that in sodium hydroxide solutions.

The effect of calcium and sodium chlorides on swelling in calcium hydroxide solutions

The addition of calcium chloride increased the swelling in calcium hydroxide solutions at all pH values (Fig. 2), the presence of 0.09M-calcium

Fig. 2. The effect of addition of CaCl_2 on the swelling of collagen in $\text{Ca}(\text{OH})_2$ solutions ($\triangle-\triangle$, no CaCl_2).

chloride slightly increased the swelling, and as the concentration was further increased the swelling progressively increased until in the presence of 1.0M-calcium chloride it was more than twice that in the hydroxide alone. The addition of a salt must decrease the swelling pressure, and the fact that calcium chloride increased swelling indicates that it must have a very marked effect in decreasing the cohesion of the collagen.

The addition of sodium chloride also increased swelling in calcium hydroxide solutions (Figs. 3 and 4). As low a concentration as 0.001 M-sodium chloride

caused a small increase in swelling, and with 0.02M-sodium chloride the swelling was approximately doubled. The swelling continued to increase until the concentration reached 0.1M and then remained approximately constant as the concentration was further increased to 0.2, 0.5 and 1M. Above 0.1M

A change in pH from 11.5 to 12.5 involves a tenfold change in the calcium-ion concentration, and since the sodium-ion concentration is constant and low, it is possible that such a rise may increase the $\text{Ca}^{++}/\text{Na}^{+}$ ratio sufficiently to cause a decrease in the swelling.

DISCUSSION

On the basis of the Donnan theory of membrane equilibria it might be expected that the swelling pressure and hence the amount of swelling would be the same with all three alkaline earth bases, and rather more than half that in sodium hydroxide solutions at corresponding pH values, the ratio of the membrane potentials in uni- and bi-valent systems being 0.66 (Bolam, 1932). Only with barium hydroxide, however, did the swelling approach half that found with sodium hydroxide and with calcium hydroxide it was very much less. The low amount of swelling in calcium hydroxide is the more surprising when it is remembered that the second dissociation constant of calcium hydroxide is comparatively low, 0.031 (Davies, 1938), so that about one-third of the calcium functions as a monovalent cation, whereas with barium hydroxide the corresponding dissociation constant is 0.23 (Davies, 1939) and nearly all the barium will be present as the divalent cation.

The unexpectedly low swelling in solutions of bivalent bases, especially calcium hydroxide, may be due to their having a smaller effect on the cohesion of the protein salts. In view of the relative effects of calcium and sodium chlorides on swelling in calcium hydroxide (Fig. 2), and in sodium hydroxide (Bowes & Kenten, 1950*a*), respectively, and of the lowering of the shrinkage temperature caused by calcium chloride (Theis & Steinhardt, 1942), it would appear that the calcium ion reduces the cohesion of the protein to a much greater extent than the sodium ion and the first suggestion is, therefore, improbable. There is a considerable amount of evidence pointing to the combination of alkaline earth cations, especially calcium, with proteins (Greenberg, 1944) and by analogy with the dissociation constants of calcium and barium salts of the hydroxy and dicarboxylic acids (Cannan & Kibrick, 1938), calcium is likely to have a greater affinity than barium for proteins. The present observations suggest that the affinity of calcium, strontium and barium for collagen decreases in the order $\text{Ca} > \text{Sr} > \text{Ba}$. No evidence is at present available regarding the relative effect of these three cations on cohesion.

It seems that three factors participate in determining the effect of additions of sodium chloride to solutions of calcium hydroxide (or of any univalent salt to a solution of a bivalent base) on the swelling of collagen: (1) The effect of the univalent cation in increasing the swelling pressure. (2) The effect of

Fig. 3. The effect of addition of NaCl on the swelling of collagen in $\text{Ca}(\text{OH})_2$ solutions.

Fig. 4. The effect of addition of NaCl on the swelling of collagen in $\text{Ca}(\text{OH})_2$ solutions.

the shape of the curve began to change, the flattening in the pH range 12.0–12.5 was less marked and the curve tended to descend more sharply with decrease in pH until it became concave in shape. Similar changes in the shape of the swelling curves in sodium hydroxide solutions with increasing sodium chloride concentration have already been described (Bowes & Kenten, 1950*a*).

The maximum shown in the neighbourhood of pH 11.5 with 0.02 and 0.04M-sodium chloride may be due to changes in the calcium-ion concentration.

the salt in decreasing the swelling pressure. (3) The effect of the salt on cohesion. The curves shown in Fig. 3 suggest that at low concentrations of sodium chloride, increase in the sodium-ion concentration is the predominant factor, the rise in the $\text{Na}^+/\text{Ca}^{++}$ ratio increasing the swelling pressure with the result that swelling increases. As the concentration of sodium chloride increases, the effect of salt in depressing the swelling pressure becomes apparent, and balances the effect of increase in the sodium-ion concentration, so that the swelling remains constant. The change in the shape of the curve above 0.1M suggests that cohesion is beginning to be affected; at concentrations of this order the swelling pressure probably begins to decrease, but the decrease in cohesion balances this and the swelling remains unchanged. At concentrations of 2M or greater, sodium chloride begins to decrease the swelling again, probably by an action similar to that occurring in the salting out of proteins (Cohn & Edsall, 1943).

SUMMARY

1. The swelling of collagen in solutions of bivalent bases was found to decrease in the order barium hydroxide $\geq$ strontium hydroxide $>$ calcium hydroxide. Swelling in barium hydroxide solutions was rather less than half, and that in calcium hydroxide solutions about one-eighth of that in sodium hydroxide solutions. The small amount of swelling in calcium hydroxide solutions is attributed to combination of calcium ions with the collagen.

2. The addition of calcium or sodium chlorides increased swelling in calcium hydroxide solutions. The increase with calcium chloride is attributed to decrease in the cohesion of the collagen, and the increase with sodium chloride to the addition of a univalent cation and consequent increase in swelling pressure.

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The Separation of the C_{12} - C_{18} Fatty Acids by Reversed-phase Partition Chromatography

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Work in this laboratory (Pollock, Howard & Boughton, 1949) on inhibition and promotion of bacterial growth by fatty acids revealed the need for analytical and preparative methods adapted to handling small quantities of these substances. Chromatography seemed to offer a possible solution to these problems.

Early attempts at adsorption chromatography showed little promise until Claesson (1946) and Holman & Hagdahl (1948, 1949) developed frontal and displacement analysis methods. These, though very successful, require rather elaborate apparatus and larger amounts of material than we wished to work with. We turned, therefore, to partition chromatography.

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This method had already been successfully applied to the separation of the lower fatty acids (Elsden, 1946; Ramsey & Patterson, 1945; Peterson & Johnson, 1948; Moyle, Baldwin & Scarisbrick, 1948). Ramsey & Patterson (1948) have extended the method to the longer chain (C_{11} - C_{19}) acids using 2-aminopyridine and furfuryl alcohol as their solvent system. They were able to separate acids differing by two CH_2 groups. It seems improbable that suitable solvent systems of this type could be found for much longer-chain fatty acids or for lipophilic substances lacking acidic groups. These substances would have, in general, partition coefficients too much in favour of the less polar phase. We tried, therefore, to evolve a partition chromatogram in which the less polar phase is stationary. The choice of solvent systems