THE RÔLE OF CARBONIC ANHYDRASE IN CERTAIN IONIC EXCHANGES INVOLVING THE ERYTHROCYTE*

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There has been described elsewhere (1, 2) a permeability phenomenon analogous in certain respects to chemical catalysis but involving a diffusion process rather than a chemical reaction. It now appears that this phenomenon of "catalyzed diffusion," as it may be called for brevity, is of wider applicability than was at first suspected. The present paper deals with certain recently investigated aspects of this question.

The work had its origin in the observation of Ørskov (3, 4) that the rate of hemolysis of mammalian erythrocytes in solutions of ammonium chloride can be enormously increased—that is, 50 times or more—by the addition of a little bicarbonate. Ørskov in his first paper (3) suggested that carbonic acid has a specific effect of some sort on the erythrocyte which makes it more permeable to anions; later (4) he modified this view and postulated instead an increased permeability of the cell to the ammonium ion. For various reasons which have in part been set forth elsewhere (5) neither of these explanations seems to be satisfactory, and there has been proposed in their place the principle of catalyzed diffusion (2), in which the action of the bicarbonate is believed to be not primarily upon the cell at all but rather upon the solutions on the two sides of the cell membrane.

In Fig. 1 A is represented the mechanism (6) for the entrance of NH_4Cl into the erythrocyte, which in spite of certain objections (3, 4) that we believe to be answerable (5), seems best to explain the known facts. According to it NH_3 molecules first enter the cell and there become converted into NH_4 ions; a subsequent shift of anions through the anion-permeable membrane completes the process. The overall rate of transfer of the salt is slow, chiefly because of the extremely low concentration of OH' ions in the cell at any given time.

If, now, NH_4HCO_3—or any other bicarbonate—be added to the external solution, we have the conditions represented in Fig. 1 B in which a new molecule of very great penetrating power, CO_2, is present. Mass law considerations demand that for equilibrium the product $[NH_4]_{inside} \times [HCO_3]_{inside}$ must

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equal $[NH_4]_{outside} \times [HCO_3]_{outside}$. Since $[NH_4]_{outside}$ is initially much higher than $[NH_4]_{inside}$ because of the high external concentration of NH_4Cl, there will be a tendency to force $[HCO_3]_{inside}$ above $[HCO_3]_{outside}$. This, however, in an anion-permeable cell would lead to an exchange of HCO_3' for Cl'. As a final result of these two processes NH_4Cl has entered the cell and

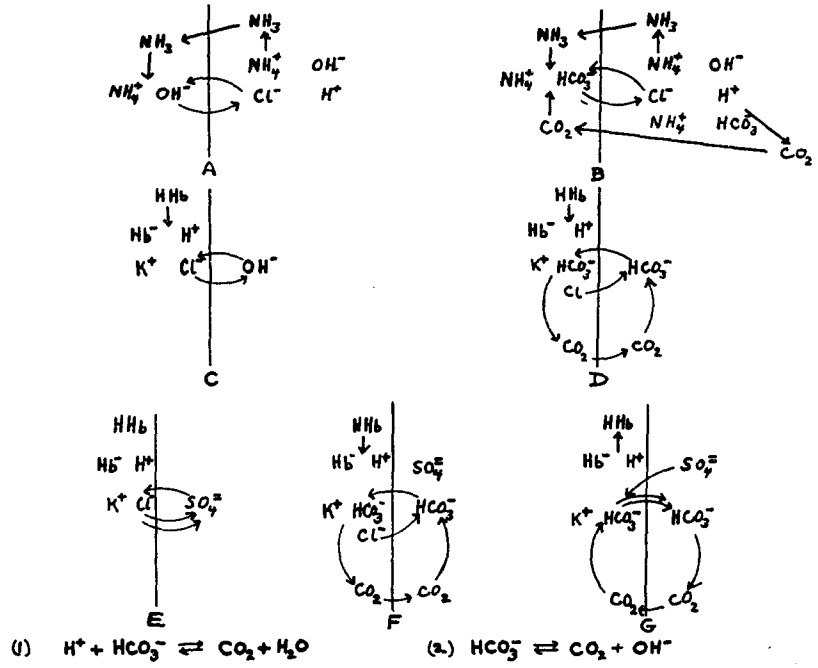

FIG. 1. Ionic exchanges involving the erythrocyte. The cell in each case is supposed to be on the left-hand side of the vertical line. (A) Cells in a solution of NH_4Cl. (B) Same, after the addition of NH_4HCO_3. (C) Cells in an alkaline bicarbonate-free solution. (D) Same, after the addition of bicarbonate. (E) Cells in a solution of a sulfate. (F) Same, after the addition of bicarbonate (first stage). (G) Same as F (second stage).

HCO_3' is back again on the outside ready to repeat the cycle. It therefore serves as a catalyst, so to speak, to get the NH_4Cl into the cell more rapidly than it could enter by mechanism 1 A.

Experimental evidence for the cycle suggested in 1 B is provided by the action of certain narcotics which considerably decrease the permeability of the erythrocyte to anions, while having little effect on that to the molecules CO_2 and NH_3. The form of the swelling curves in the presence of such substances is, in general, that demanded by the theory (2). Low concentrations of tannic acid may also be used to separate in part the "molecular" from the "ionic"

portions of the swelling processes. More direct evidence in favor of the catalyzed diffusion theory, however, is furnished by another method, entirely different in principle, with which the present paper is chiefly concerned.

II

It will be noted that in the cycle represented in Fig. 1 B there is at one point a conversion of CO_2 to HCO_3'; at another point the reverse change from

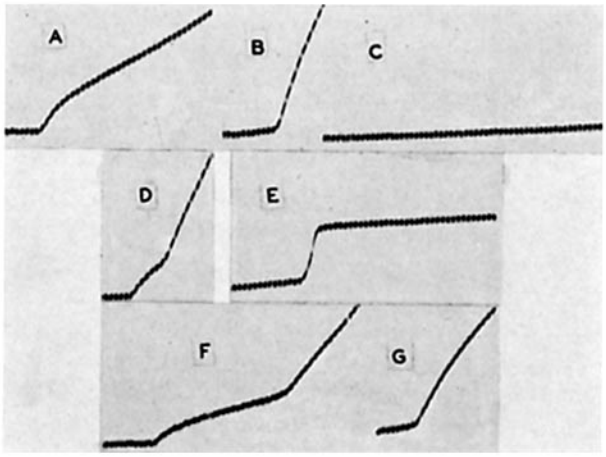

FIG. 2. Volume changes of washed beef erythrocytes in M/6 NH_4Cl to which NH_4HCO_3 is added. 22°C. The breaks in the curves indicate intervals of 1 second. (A) 0.003 M NH_4HCO_3 added. (B) Same, with carbonic anhydrase in the external solution. (C) Same as B in the presence of 125 mg. per cent of sulfanilamide. (D) Same as A, but carbonic anhydrase added 6 seconds after the bicarbonate. (E) Same as B, but KCN (0.007 M) added a few seconds after the bicarbonate. (F) Same as D, but with half as much bicarbonate; enzyme added after about 25 seconds. (G) Same as F, but enzyme added before the bicarbonate.

HCO_3' to CO_2 occurs. Both of these changes are known in themselves to be rather slow, but to be greatly accelerated by the enzyme carbonic anhydrase (7). If, therefore, the catalyzed diffusion theory be true, the overall rate of the entire process should be strongly influenced by this enzyme; according to the purely static theory of Ørskov, on the other hand, there is no very obvious reason why such an enzyme should have any effect. The experiments here to be reported, involving in part the addition of more carbonic anhydrase to the hemolytic system and in part the inactivation of that already present in the erythrocytes, have given results which strongly support the theory of catalyzed diffusion.

In Fig. 2 are represented certain volume changes of beef erythrocytes, re-

corded photographically by the method of Parpart (8). The breaks in the curves indicate intervals of 1 second. In Fig. 2 A washed beef cells were placed in M/6 NH_4Cl, in which they swell very slowly. At the point where the sudden rise of the curve begins 0.1 ml. of M/2 NH_4HCO_3 was added to 15 ml. of the suspension, the resulting bicarbonate concentration therefore being approximately 0.003 M. The swelling of the cells shown in the photograph passed imperceptibly into hemolysis, which under these conditions required several minutes for its completion, but which with the scale of magnification here used could not be recorded in full on the photographic paper.

The conditions in Fig. 2 B were exactly the same as in Fig. 2 A except that at the beginning of the experiment one drop of a solution containing carbonic anhydrase prepared by the method of Meldrum and Roughton (7) had been added to the cell suspension a few seconds before the beginning of the photographic record. It is obvious that the swelling process was strikingly accelerated; the same was true of the hemolysis which followed. An interesting modification of the experiment is shown in Fig. 2 D in which the enzyme was added after the bicarbonate instead of before it. The almost instantaneous acceleration of the swelling process which it produced is clearly shown. Figs. 2 F and 2 G correspond exactly to Figs. 2 D and 2 B, respectively, except that the concentration of bicarbonate here employed was only half as great, and the rate of swelling both before and after the addition of the enzyme was in consequence considerably slower. The addition of enzyme inactivated by heat had no such effect.

An experiment of the opposite sort is illustrated in Fig. 2 C; in it the enzyme within the cells as well as that added externally in the same amount as in Fig. 2 B had been inactivated before the addition of the bicarbonate by means of sulfanilamide (125 mg. per cent). This substance is known to have a specific inhibitory action on carbonic anhydrase (9). It will be seen that under these conditions the bicarbonate effect almost disappeared. The same general result has been obtained many times under a variety of conditions. It may be noted that the effect of sulfanilamide on the permeability of the erythrocyte to various non-electrolytes such as glycerol or thiourea or even to ammonium salts such as acetate, which do not require an ionic exchange for their penetration (6) is comparatively slight.

While sulfanilamide acts almost instantaneously on the carbonic anhydrase in the external solution, its effect in the interior of the cell is subject to a slight delay. Thus the rate of swelling, on the addition of bicarbonate, of cells suspended in NH_4Cl saturated with sulfanilamide is distinctly slower after an exposure of 2 minutes than after one of a few seconds; indeed, in the latter case a complete photographic record shows for a short time a gradual decrease in the rate of swelling, after which no further change of rate occurs. Conversely, cells previously saturated with sulfanilamide do not instantly reach their

maximum rate of swelling when placed in a sulfanilamide-free mixture of NH_4Cl and NH_4HCO_3. It may be concluded, therefore, that although in its powers of entering living cells sulfanilamide must be classed as a readily penetrating compound, the erythrocyte is not as permeable to it as it is, for example, to urea, acetamide, various alcohols, etc.

It is also known that carbonic anhydrase can be reversibly inactivated by cyanides (10). The rapidity with which these substances produce an internal effect on the erythrocyte is well shown in Fig. 2 E, in which the addition of 0.05 ml. of 2 M KCN to 15 ml. of the stirred cell suspension almost instantly stopped even the maximum rate of swelling attainable under the conditions of the experiment. The inhibitory effect of cyanides on the enzyme present in the interior of the erythrocyte seems to be completely reversible on washing the cells. It should be noted that while cyanides inhibit the catalytic effects of bicarbonates on the entrance of NH_4Cl, they may under appropriate conditions have a catalytic effect of their own, though a considerably weaker one than bicarbonates. This behavior would be expected from certain other similarities between carbonic acid (11) and HCN (12) and their salts. An analogous effect of sulfides might also be anticipated (13) and has in fact been indicated by some preliminary experiments (2).

The effects of carbonic anhydrase and of sulfanilamide on the Ørskov effect can also readily be demonstrated and even measured quantitatively with a very satisfactory degree of accuracy by the hemolysis method, which in its simplest form requires no apparatus but a test-tube and a stop-watch. This method, in general, gives results very similar to those already described except that in any given experiment only a single point on the swelling curve instead of the entire curve is open to investigation.

To meet the possible objection that the supposedly pre-hemolytic optical changes recorded in Fig. 2 might be due in part to some undetected non-osmotic type of hemolysis, the experiment was varied so as to avoid hemolysis altogether. In Fig. 3 are shown the volume changes of erythrocytes suspended in isotonic NaCl when shrinkage is first produced by the addition of saturated NH_4Cl (0.3 ml. to 15 ml. of suspension) and restoration of the original volume is then accelerated by the further addition, at the point where the slope of the curve changes, of 0.1 ml of M/2 NH_4HCO_3. The rise of the curves somewhat above the starting point in these figures is due to the unavoidable slight dilution of the cell suspension, to which the optical system used for recording is very sensitive. As before, the acceleration of the rate of swelling by bicarbonate (Fig. 3 A), the further acceleration by the addition of enzyme (Fig. 3 B), and the almost complete abolition of the bicarbonate effect by sulfanilamide (Fig. 3 C) are clearly shown.

The effect of sulfanilamide under these various conditions is readily reversible. Even after an exposure of more than 30 hours to a saturated solution

in isotonic NaCl beef erythrocytes recovered their original behavior after being washed twice in a sulfanilamide-free isotonic salt solution. As to the lowest effective concentration of sulfanilamide, the hemolysis method with human erythrocytes under certain conditions (pH 7.6 with a phosphate-phthalate buffer) showed a distinct inhibiting action of as little as 0.03 mg. per cent, which is approximately the same as the minimum concentration (2×10^{-6} M) found by Mann and Keilin (9). We have some reason to believe, however, that a systematic search for the optimum experimental conditions might enable this value to be carried even lower. As it stands, it is roughly only 1/300 of a

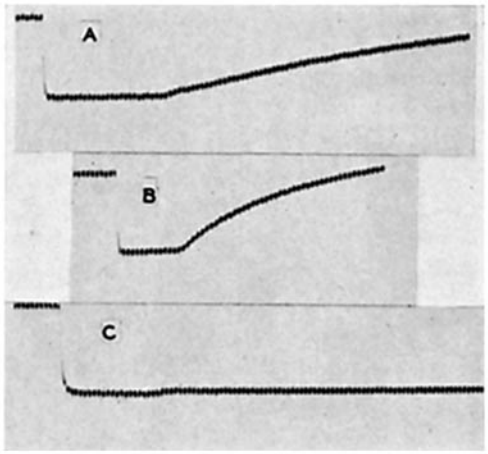

FIG. 3. Volume changes of washed beef erythrocytes originally in M/6 NaCl. 0.3 ml. of saturated NH_4Cl added to 15 ml. of the suspension, followed by 0.05 ml. of M/2 NH_4HCO_3. 22°C. The breaks in the curves indicate intervals of 1 second. (A) NH_4HCO_3 alone added. (B) Same as A, but with carbonic anhydrase in the external solution. (C) Same as B in the presence of 125 mg. per cent sulfanilamide.

typical concentration (10 mg. per cent) in the blood of human patients under sulfanilamide therapy. In this connection it may be mentioned that sulfapyridine, as would be anticipated from its behavior with carbonic anhydrase *in vitro* (9), has been found to have no demonstrable inhibitory effect on the volume change of erythrocytes produced by NH_4Cl—NH_4HCO_3 systems.[1]

III

As a result of the experiments so far outlined and others of a similar nature it may be said with some confidence that the Ørskov effect is not only influenced by carbonic anhydrase but is largely dependent on it. Support therefore seems to be given to the catalyzed diffusion theory. The principle in question, has,

[1] Sulfathiazole behaves like sulfapyridine.

however, a wider applicability than merely to the behavior of ammonium salts, since it seems to be involved in other more frequently encountered ionic exchanges of a different sort.

Consider, for example, the volume changes of erythrocytes produced by pH changes of the external medium. These cells have long been known to swell in acid and to shrink in alkaline solutions (14–16) at rates which are, in general, rather slow, requiring minutes or possibly sometimes even hours for the attainment of the final equilibrium volume. In the presence of a little bicarbonate, however, we have found that the corresponding times may, under favorable conditions, be shortened to a few seconds; and this fact has since been put to practical use in bringing about rapid equilibration of erythrocytes to solutions of altered pH.

The theory of the effect is very simple (Fig. 1 C). On making an external bicarbonate-free solution more alkaline there is an exchange of OH' ions from the solution for Cl' ions from the cell. Most of the OH' ions combine within the cell to form water, which is osmotically inactive, the $K^{\cdot}$ formerly paired with the Cl' now being held by the polyvalent hemoglobin ion. The osmotic effect of the Cl' is therefore largely lost without being replaced by anything else, and the cell shrinks. The rate of the process, except at high degrees of alkalinity, is quite slow, presumably because of the low concentration of OH' ions. If, however, bicarbonate be present (1 D) in a concentration which, though low as compared with that of Cl', is very high as compared with that of OH', a rapid "catalytic" cycle becomes possible between the more alkaline external solution and the more acid cell. On the acid side of the cell membrane the tendency is to form CO_2 from HCO_3'; on the alkaline side, the reverse. As long as a pH difference exists between the cell and its surroundings and there is Cl' to exchange for HCO_3' the cycle can continue (see in this connection 17). The exchange of Cl' for HCO_3' is known to be an extremely rapid process (18, 19), and in the presence of carbonic anhydrase the other parts of the cycle can also become rapid. The final equilibrium should therefore be approached far more quickly in the presence than in the absence of bicarbonate.

It is easy to show the effect of bicarbonates and of carbonic anhydrase on the rates of shrinkage and swelling produced by pH changes. In Fig. 4 A beef erythrocytes were suspended in a buffered salt solution at pH 7.01. Enough NaOH was then added to change the pH to 8.35. The shrinkage that followed was so slow that no attempt was made to follow it photographically to its end. Instead, after about 90 seconds enough acid was added approximately to restore the original pH; and a slow return towards the original volume occurred. It will be seen in Fig. 4 B that the presence of a little bicarbonate greatly accelerated both processes, particularly the swelling. The addition of carbonic anhydrase (Fig. 4 C) still further increased the rapidity of shrinkage, though under the conditions of this particular experiment it did

not make very much difference in the swelling process. In the presence of sulfanilamide, on the other hand (Fig. 4 D), the same amounts of bicarbonate and of enzyme as in Fig. 4 C were almost without effect.

Particularly striking results can be obtained with cells suspended in isotonic sugar solutions made alkaline with NaOH. Under these conditions shrinkage

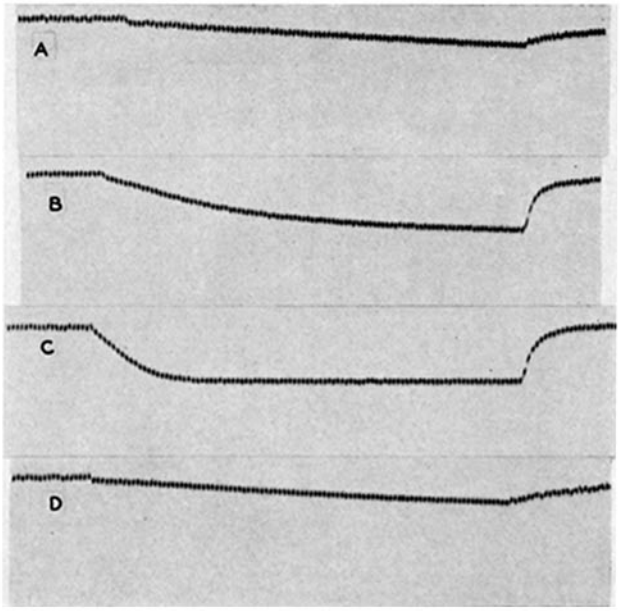

FIG. 4. Volume changes of washed beef erythrocytes originally in M/6 NaCl buffered at pH 7.01 with a phosphate-phthalate mixture. 22°C. The breaks in the curves indicate intervals of 1 second. (A) Sufficient NaOH added to change the pH to 8.35, then sufficient HCl approximately to restore its original value. (B) Same as A in the presence of 0.002 M $NaHCO_3$. (C) Same as B with carbonic anhydrase in the external solution. (D) Same as C in the presence of 125 mg. per cent of sulfanilamide.

occurs fairly rapidly, but can be greatly accelerated by bicarbonates. Fig. 5 A represents the behavior of a stirred suspension of beef erythrocytes in a 0.3 M sucrose solution which, at the point where the descent of the curve begins, was suddenly brought to pH 8.9 with NaOH. The resulting shrinkage of the cells was still in progress, though nearly complete, in 90 seconds. Fig. 5 B represents a similar experiment, begun in exactly the same way, but with the addition after 15 seconds of a small amount of bicarbonate (0.002 M). The response of the cells to the presence of the bicarbonate is strikingly shown. A second addition of the same amount of bicarbonate after the descent of the

curve had been completed (6 seconds from the end of the record) can be seen to have had almost no further effect. The pH of the solution in this case was little changed by either addition of bicarbonate, the chief effect of the latter being greatly to hasten the attainment of an otherwise predetermined equilibrium—which is the essence of the behavior of a catalyst.

Another series of experiments, not here represented by photographs illustrate another aspect of the "catalytic" effect of bicarbonates. In each case

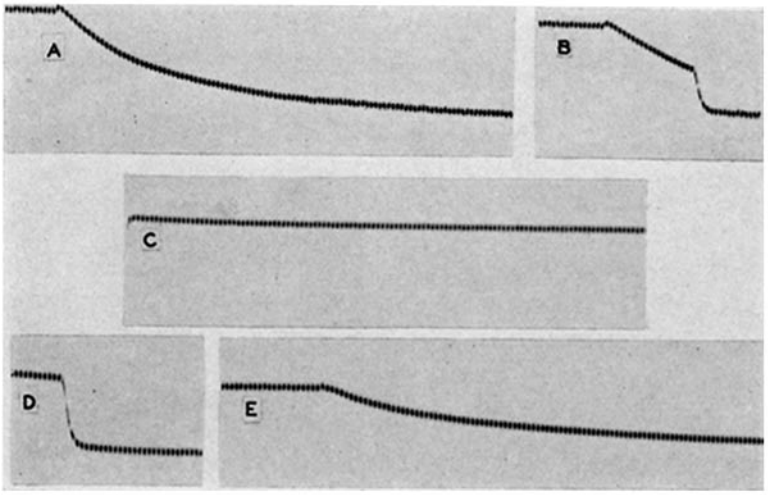

FIG. 5. Volume changes of washed beef erythrocytes originally in 0.3 M sucrose. 22°C. The breaks in the curves indicate intervals of 1 second. (A) Sufficient NaOH added at the descent of the curve to bring the pH to 8.9. (B) Same, but at the point of the second, more rapid descent $NaHCO_3$ (0.002 M) was added. An equal amount of $NaHCO_3$ was again added 6 seconds from the end of the record. (C) Cells in 0.3 M sucrose. (D) Same as C but at the point of the rapid descent $NaHCO_3$ (0.002 M) was added. (E) Same as D in the presence of 125 mg. per cent of sulfanilamide.

the cells were placed in a 0.3 M sucrose solution made alkaline with NaOH, and bicarbonate was added as before, but after different times in the different experiments. (See also in this connection Fig. 6.) The rapid shrinkage induced by this addition was very different in magnitude according to the time at which it occurred, but in each case was approximately that required to complete the process of shrinkage already in progress. As before, a further addition of bicarbonate after the attainment of the final cell volumes had only negligible effects.

Bicarbonate effects of this type, like those involving ammonium salts, are greatly reduced by inhibitors of carbonic anhydrase such as cyanides and sulf-

anilamide, as is shown in the series of experiments represented in Fig. 5. In Fig. 5 C washed beef erythrocytes were suspended in 0.3 M sucrose, in which a very slow shrinkage takes place, presumably in consequence of the exchange of Cl' ions from the cell for OH' ions from the surrounding non-electrolyte solution (for a discussion of this behavior, see Jacobs and Parpart, 20, 21, and Wilbrandt, 22). The addition of bicarbonate to such a suspension has the double effect of greatly increasing the concentration of OH' and at the same

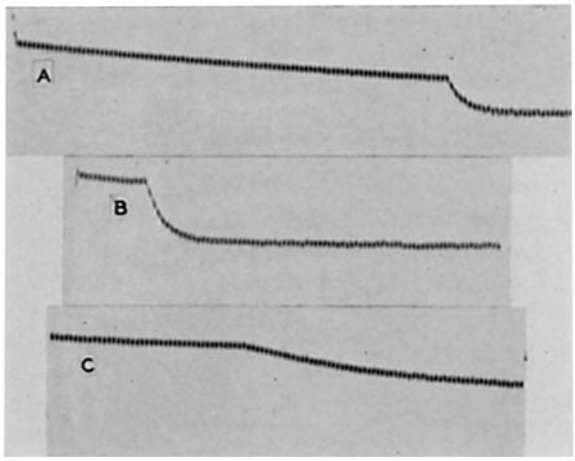

FIG. 6. Volume changes of washed beef erythrocytes in M/12 Na_2SO_4 buffered at pH 7.4. (The slight hypotonicity of the solution was intentional to avoid the possibility of direct osmotic effects during the course of the experiment in the same direction as those caused by the ionic exchange.) 22°C. The breaks in the curves indicate intervals of 1 second. (A) At the point of sudden descent of the curve $NaHCO_3$ (0.002 M) was added. A second equal amount added a few seconds from the end of the curve had a negligible effect. (B) Same as A, but the first addition of bicarbonate was made earlier. (C) Same as B in the presence of 125 mg. per cent of sulfanilamide.

time bringing into action the catalytic mechanism already discussed. The resulting shrinkage (Fig. 5 D) was the most rapid that has as yet been obtained by any method involving the effects of increased alkalinity, being virtually complete in 4 or 5 seconds. The effect of sulfanilamide, shown in Fig. 5 E, is greatly to retard this change, which in its presence was not finished even at the end of 90 seconds. It should, of course, be noted in this, as in other cases that bicarbonates are not completely ineffective in the absence of active carbonic anhydrase, since the reaction $CO_2 + H_2O \rightleftarrows H_2CO_3$ can take place in either direction without the assistance of any enzyme, though at a greatly reduced rate.

The general conclusion to be drawn from the experiments discussed in the present section, and from other similar ones that have not been mentioned, is that the "catalytic" effect of bicarbonates on the alterations of the cell volume induced by pH changes is as striking as that previously described for ammonium salts. Like the latter, it is dependent for its full effectiveness on the presence of the enzyme carbonic anhydrase.

IV

In the cases so far discussed the rôle of CO_2 and HCO_3' in promoting ionic exchanges is fairly obvious. A somewhat more surprising instance of an apparent catalytic effect of bicarbonates has been found in connection with the volume changes of erythrocytes associated with the exchange of Cl' from the cells for SO_4'' from the surrounding solution. This phenomenon, which depends on the fact that one bivalent ion is osmotically less effective than the two univalent ions for which it must be exchanged, has recently been studied by Parpart (23), who worked exclusively with well washed erythrocytes from which most of the bicarbonate had presumably been removed. Under these conditions he found that the rate of exchange of the two ions is rather slow, the times for one-half completion of the process at 20°C. frequently being measured in minutes rather than in seconds.

In repeating some of Parpart's experiments with M/9 and M/12 solutions of Na_2SO_4 buffered at different pH values by a phosphate-phthalate mixture, we were surprised to find that the addition of low concentrations of sodium bicarbonate rapidly brought about the attainment of the final volume—frequently within a few seconds. That the effect was not merely that of alkalinity *per se*, which Parpart had studied, was shown by the fact that the pH changes of the buffered solution produced by low concentrations of bicarbonate were usually rather slight. Furthermore, even with the most careful pH control the bicarbonate was found to have a relatively enormous effect of its own, which in several important respects resembled that described in the preceding sections.

For example, at a constant pH value the cell volume finally attained in a buffered solution of Na_2SO_4 was, within reasonable limits, independent of the amount of bicarbonate added, though the rates of its attainment varied as before. Furthermore, at constant pH, the addition of more bicarbonate after the equilibrium volume had been reached had little effect. Likewise, the magnitude of the acceleration following the addition of the bicarbonate depended on the time of its addition. Finally, and perhaps most important, the bicarbonate effect largely disappeared in the presence of sulfanilamide or KCN, indicating that, as before, the enzyme carbonic anhydrase was playing a part in the process. Some of these peculiarities of behavior are shown in Fig. 6.

Though the exact mechanism of action of HCO_3' on the Cl'-SO_4'' exchange is still under investigation, a tentative explanation of it may be suggested. As has already been noted above, the exchange of HCO_3' for Cl' is known to be extremely rapid. When, therefore, sodium bicarbonate is added to a solution of a salt with a much more slowly penetrating anion such as SO_4'' there will be a tendency, regardless of this ion, for an immediate approach toward the Donnan distribution $[Cl']_i/[Cl']_o = [HCO_3']_i/[HCO_3']_o$, the subscripts i and o indicating inside and outside respectively. It is easy to show that for a ratio of volume of solution to volume of cells of 600:1, which was approximately that involved in the present experiments, an exchange of equal numbers of the two ions in question would lead to a very high value of $[HCO_3']_i$ as compared with $[HCO_3']_o$, *i.e.*; to a considerably increased internal alkalinity of the cell. This, by the mechanism already discussed, would lead to a rapid shrinkage of the cell; and it is reasonable to suppose that the immediate volume changes on the addition of bicarbonate recorded in Fig. 6 may be largely due to this factor. Indeed, a situation in which it is the only factor of significance is provided by the use of isotonic sodium citrate instead of sodium sulfate for the external solution. Since erythrocytes are almost impermeable to the citrate ion (2, 24) their behavior in a solution of sodium citrate, both before and after the addition of bicarbonate, in certain respects should be, and has actually been found to be, essentially the same as that in a solution of sucrose, which has already been discussed.

The subsequent exchange of SO_4'' for HCO_3' cannot be followed by the method of volume changes, since theoretically about the same shrinkage should result either from the exchange of a given number of Cl' ions from the cell for an equal number of HCO_3' ions, or for half the number of SO_4'' ions, from the surrounding solution. It has, in fact, been found experimentally that cells first equilibrated with M/6 $NaHCO_3$ and then placed in M/9 Na_2SO_4 show an almost complete absence of the moderately slow volume changes that occur under these conditions with cells previously equilibrated with M/6 NaCl.

While the most obvious part of the volume change produced by the addition of bicarbonate to a Na_2SO_4 solution therefore appears to be associated with a temporary tendency towards increased intracellular alkalinity (Fig. 1 F), it would seem theoretically that under the conditions of these experiments SO_4'' ought to enter the cell more rapidly after most of the Cl' of the cell has been replaced by HCO_3' than before, in consequence of the operation of the cycle represented in Fig. 1 G. Some experimental evidence in favor of this view has been obtained by studying the increases in cell volume on the addition of small amounts of NaCl to the external solution at different times during and after the shrinkage process; but more work along these lines will be required before this evidence can be considered complete. There is also a

possibility, suggested by the work of Parpart, that the increased intracellular alkalinity resulting from the exchange of Cl' for HCO_3' may make the cell more permeable to the SO_4'' ion. At all events, there is no doubt that the otherwise rather slow shrinkage of erythrocytes in buffered Na_2SO_4 solutions can be greatly accelerated by the presence of low concentrations of bicarbonates, this effect being, in turn, strongly influenced by the enzyme carbonic anhydrase.

What is true of the erythrocyte is doubtless also true of other cells which are permeable to anions. The presence of carbonic anhydrase in large quantities in tissues such as the gastric mucosa (25), the gills of fishes (26), etc., in which there is reason to believe that exchanges of anions of different sorts takes place, is of possible interest in this connection. In conclusion, it may be noted that in the case of cells which are more permeable to cations than to anions, of which many examples are known in both animals and plants, it would be reasonable to expect that ammonia might perhaps take part in a "catalytic" cycle similar to that here suggested for carbonic acid in the case of anion-permeable cells (27). This field has, however, not yet been investigated.

SUMMARY

1. The acceleration by bicarbonates of the swelling and hemolysis of erythrocytes in solutions of ammonium salts, first reported by Ørskov, is strikingly dependent upon carbonic anhydrase, being almost abolished by inhibitors of this enzyme such as KCN and sulfanilamide, and under suitable conditions being enhanced by its addition to the external solution. This behavior gives support to the theory of "catalyzed diffusion" as an explanation of the Ørskov effect.

2. The inhibitory effects of both sulfanilamide and KCN seem to be capable of complete reversal on washing the erythrocytes in isotonic salt solutions. The full effect of KCN appears almost instantly; that of sulfanilamide requires a period measured in seconds, or possibly even in minutes, to reach its maximum, the delay presumably being due to the slower penetration of the erythrocyte by this substance. Under favorable conditions the effect of concentrations of sulfanilamide of a few hundredths of a milligram per cent can be demonstrated. No similar effects have been obtained with sulfapyridine.

3. Bicarbonates also have a "catalytic" effect on the response of the internal pH of erythrocytes to changes in that of their surroundings. The resulting volume changes of the cell, which otherwise frequently require many minutes for their completion, may take place within a few seconds in the presence of low concentrations of bicarbonates. At a given pH value the effect of the latter substances is chiefly on the rate of the change and only to a minor extent on its magnitude. It may be further accelerated under appropriate conditions

by the addition to the cell suspension of carbonic anhydrase, and can be almost abolished by KCN and by sulfanilamide.

4. Volume changes of erythrocytes associated with exchanges of Cl' for SO_4'' ions are greatly accelerated by low concentrations of bicarbonates, this effect being likewise dependent upon carbonic anhydrase. There is some evidence that in this case the exchange takes place, at least in part, in two steps: Cl' for HCO_3' and HCO_3' for SO_4''.

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