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The Effect of the Bicarbonate Ion on the Gallbladder Salt Pump

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Summary. The salt pump of the isolated rabbit gallbladder, which transports an isotonic fluid solution from the lumen of the organ to the serosal tissue spaces, utilizes an endogenous substrate source for the neutral transport of NaCl. Exogenous substrates (glucose, acetate) do not elevate transport rates above control values if added when the preparation is made, but exogenous substrates are utilized, as pumping is maintained at initial pumping rates for longer periods of time than without substrate. Substrate addition with the inclusion of bicarbonate ion is a stabilizing factor for this *in vitro* preparation. Although bicarbonate ion leads to an increase in transport rate without exogenous substrate, the pumping rate declines with time. Oxygen consumption falls over long periods with continued bicarbonate presence. The bicarbonate effect persists in the *in vitro* preparation after 20 to 24 hr at 4 °C. After such treatment, glucose stimulates fluid transport. Thus, bicarbonate ion alters the glucose utilization of the gallbladder mucosa and the channeling of energy for fluid transport.

Bicarbonate ion influences the rate of fluid transport across the epithelia of kidney, gut and gallbladder of mammalian tissues. The *in vitro* rat kidney slice exhibits a slower rate of transport if the bicarbonate ion is omitted than when it is present (Rumrich & Ullrich, 1968; Maude, 1970). A similar dependency of fluid transport on bicarbonate ion has been noted in the rabbit ileum (Field, Fromm & McColl, 1971) and the human jejunum (Sladen & Dawson, 1968). In the rabbit gallbladder transport is suppressed by carbonic anhydrase inhibitors (Wheeler, Ross & King, 1969) and direct addition of bicarbonate ion increases transport (Diamond, 1964).

A broad hypothesis to explore the causal relationship between bicarbonate ion effects and the active transport process is presented in the present paper. The evidence suggests that the bicarbonate ion affects one or more steps in the delivery of energy to the active transport process.

Materials and Methods

The Gallbladder Preparation

Gallbladder preparations were made by procedures similar to those described by Diamond (1964). Rabbits 3.5 to 5 kg in weight were killed by a blow on the head and the animal exsanguinated by decapitation. This procedure rendered the liver essentially free of blood and avoided the potential metabolic effects of anesthesia. After the gallbladder was cut away slightly behind the cystic duct, the bile was drained and care was taken that bile should not contact the serosal surface. The gallbladder was then transferred to a clean vessel and everted, rinsed, and again transferred to fresh Ringer's solution where re-eversion was performed. To measure the rate of fluid transport, the gallbladder was cannulated in its *in vivo* orientation and filled with fluid, the cannula was plugged, and transfer of fluid from mucosa to serosa (inside to outside) was determined gravimetrically by measuring the progressive loss of weight of the gallbladder sac at 5-min intervals (Diamond, 1964). Between weighings the gallbladder was suspended in the appropriate Ringer's solution (Table 1) which was maintained at 38 °C. To measure oxygen consumption, the organ was everted so that the transporting cells (mucosa) faced outwards.

Measurement of Oxygen Consumption

Oxygen uptake of rabbit gallbladder *in vitro* was determined by observing the rate at which oxygen tension decreased in a closed vessel containing the gallbladder. Oxygen tension (pO_2) was measured with a Clark electrode (YSI 4004). The electrode was calibrated by measuring its output when the vessel contained solutions equilibrated with air and nitrogen-oxygen mixtures (60% O_2, analyzed to within ± 0.1% O_2). The calibration was again checked in each experiment. A linear relationship between air saturation and 60% O_2 was obtained and thus a constant expressing voltage output with respect to percentage of O_2 could be calculated. A recording of voltage as a function of time could then be converted to percentage of O_2 as a function of time. The solubility coefficient for oxygen was interpolated as 0.0236 for 38 °C (Hodgman, 1958).

The fluid contents of the vessel were maintained at 38.0 ± 0.2 °C by a water jacket through which fluid was circulated by a Bronwill temperature-regulating pump. A magnetically coupled stirring bar at the bottom of the vessel insured continuous mixing. The above procedures are described in greater detail in Martin and Diamond (1966). Measurements of oxygen consumption were related to the dry weight of the gallbladder. Dry weight was obtained by drying the organ overnight in an oven at 105 °C.

Table 1. Composition of solutions (salts in mM)

Solution	NaCl	KCl	$CaCl_2$	$MgSO_4$	KH_2PO_4	K_2HPO_4	NaH_2PO_4	$NaHCO_3$
Phosphate Ringer's	142	–	2	1.2	0.375	2.125	–	–
Bicarbonate Ringer's	115	7	2	1.2	–	–	1.2	25

Bicarbonate-buffered Ringer's solution was equilibrated with CO_2 and O_2 in the ratio of 5:95.

In one set of experiments the concentration of bicarbonate was manipulated as a variable. Control of pH and the volatile buffer was achieved by mixing O_2 and CO_2 in a manifold at flow rates which would give the desired ratio of O_2 to CO_2. The gas was then passed through a gas washing bottle to saturate it with water vapor. The water-saturated gas mixture was then bubbled through a solution of 25 mM $NaHCO_3$ (38 °C) in which a pH probe was immersed. The outlet of this vessel then gassed the beaker containing the gallbladder at 38 °C. Thus, the pCO_2 of the vessel containing the gallbladder could be maintained at the proper value by maintaining the pH of the 25 mM bicarbonate at a previously determined value.

Results

Fig. 1 shows the typical time course of the rate of isotonic fluid transport in the *in vitro* rabbit gallbladder when the Ringer's solution lacks both an exogenous energy source (glucose) and the bicarbonate ion. There is an

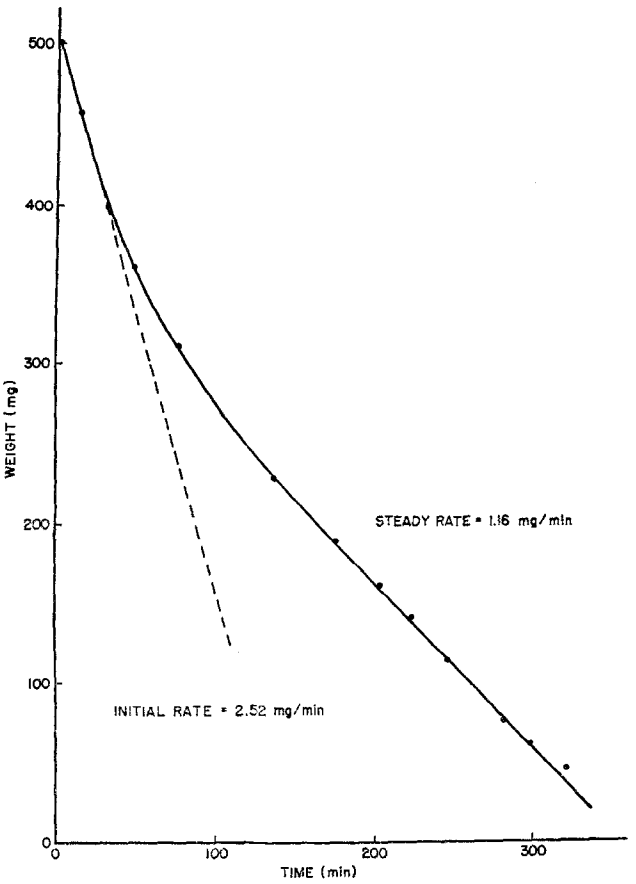

Fig. 1. Fluid transport in the isolated rabbit gallbladder. Weight loss in mg of phosphate Ringer's solution (0 mM HCO_3^-, 0 mM glucose)

Table 2. Fluid transport (μliters/mg (dry wt) hr, $\bar{x} \pm$ SE)

Substrate (mM)	n	Initial	4th hr	p*
0 mM HCO_3^-				
0	6	12.3 ± 1.7	5.22 ± 0.5[a]	0.01
Glucose (11)	5	10.3 ± 2.7	7.44 ± 2.5	0.01
Acetate (6)	5	10.6 ± 2.5	9.65 ± 2.5	
25 mM HCO_3^-				
0	6	10.9 ± 2.7	3.0 ± 0.5[b]	0.01
Glucose	6	12.0 ± 2.4	37.9 ± 12.9[c]	0.10

* Determined from the calculation of t for the paired variates of 1st hr *vs.* 4th hr. Comparisons at the 4th hr by calculating t for the difference between means. a *vs.* b, $p < 0.02$. a *vs.* c, $p < 0.05$. b *vs.* c, $p < 0.05$.

Time-dependent fluid transport rates in the *in vitro* rabbit gallbladder. The rate of fluid transport in a single gallbladder at the 4th hr is compared to the initial rate of the same gallbladder. The conditions of the experiments were the presence or absence of bicarbonate ion over the entire experimental period. Where substrates are indicated they were also present throughout the 4-hr period. For the time-dependent tests only the conditions of no bicarbonate with acetate treatment and bicarbonate presence with glucose gave rates unchanged over the 4-hr period. Comparisons of conditions and treatments at the initial time show that there are no differences. At the 4th hr, transport without glucose is slower when bicarbonate is present than in its absence. Gallbladders exposed to both bicarbonate ion and substrate maintain transport at a higher rate than do gallbladders without glucose and bicarbonate. Similarly, transport without glucose in the presence of bicarbonate is slower than the transport with both substances present.

initial rapid rate of transport which slows to a steady rate in about 2 hr. The time course of the changes in transport rate may be altered by manipulating the composition of the Ringer's solution and by the addition of exogenous substrates. Table 2 presents the results obtained when the exogenous substrates are made available in Ringer's solutions with and without the bicarbonate ion. The test for substrate effect was carried out by measuring transport in the gallbladder soon after it was removed from the rabbit and again 4 hr after the first measurement. These times of measurement correspond with the initial rate and the steady rates presented in Fig. 1. Two parameters were studied in this manner: (1) the presence or absence of the bicarbonate ion; (2) the presence of exogenous substrate under the above conditions. The statistical test was performed on the hypothesis that the rate of transport at the fourth hour was equal to the rate at the first hour. Without bicarbonate ion present acetate supported transport for the 4-hr period while glucose did not (Table 2). However, with bicarbonate ion present glucose did sustain transport throughout the experimental period. The apparent increase in transport with the addition of glucose in the pres-

ence of bicarbonate is not significant ($p < 0.10$, > 0.05) as the quantitative response was highly variable (10% to 10-fold increases). Thus, high rates of transport can be maintained over long experimental periods with the addition of glucose and bicarbonate ion together. Comparisons between conditions and treatment at the 4th hr (Table 2) show that without either bicarbonate or glucose the transport rate is sustained at a greater rate than if only bicarbonate is present ($p < 0.02$). With the glucose present and no bicarbonate, transport is again maintained at a higher rate than the case of bicarbonate without substrate ($p < 0.05$). Glucose and bicarbonate very adequately support transport ($p < 0.05$). These results indicate that in the presence of bicarbonate endogenous energy sources are utilized very rapidly for the transport function of this tissue. Moreover, this rapid utilization can be maintained with the addition of exogenous substrates.

The rate of isotonic fluid transport represents the rate of energy expenditure for one component of osmotic work. The rate of oxygen consumption represents the rate of energy conversion for all kinds of biological work. Since the fraction of the total oxygen consumption associated with isotonic fluid transport is measurable (9%, Martin & Diamond, 1966), comparison of the oxygen consumption data for gallbladders in the presence and absence of bicarbonate reflects the energy utilization between these two conditions.

Fluid transport and respiration in the same gallbladder are expressed in Fig. 2 and Table 3. In these experiments the oxygen consumption over several hours was measured with bicarbonate ion present. In another set of gallbladders the oxygen consumption was also measured when the bicarbonate ion was absent. In both series there were no exogenous substrates present. The rate of fluid transport declines as expected for all gallbladders (Fig. 1, Fig. 2*a*). However, the relative rates of metabolic activity in the two cases were different after long periods *in vitro* (Fig. 2*b*). Oxygen consumption declined (39%) in those gallbladders exposed to the bicarbonate ion, while those gallbladders which were transporting in the absence of the bicarbonate ion showed an average increase in Q_{O_2} over the initial rate (16%).

In gallbladders unexposed to bicarbonate ion it is reasonable to assume that the rate of ATP production is unchanged as the respiratory activity remains relatively constant throughout the experimental period. Since fluid transport declines significantly over this period of time one can conclude that the ATP produced was not available for transport work. That is, energy produced by mitochondrial activity is not obligatorily channeled to the transcellular ion transport mechanism. By way of contrast, the presence of the bicarbonate ion alters the metabolism of the mucosal cell in such a way

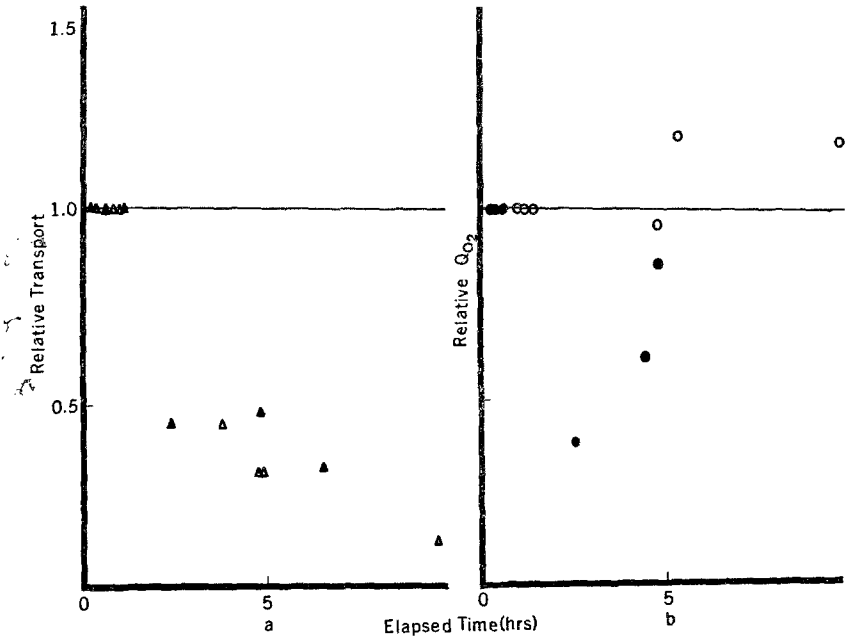

Fig. 2. Relative transport (*a*) and respiration (*b*) as a function of elapsed time (hr). Open (△) triangles are measurements of transport without bicarbonate ion present. Closed (▲) triangles are those data obtained with bicarbonate ion present. Each point is plotted as the fraction of the initial rate for an individual gallbladder. Q_{O_2} was obtained on the same gallbladders and plotted in the same fashion as the transport data. Open (○) circles, without bicarbonate; closed (●) circles, with bicarbonate. The condition of the presence or absence of bicarbonate ion was over the entire experimental period. No exogenous substrates present. Relative data derived from Table 3

as to result in a decrease in both Q_{O_2} and transport over the experimental period. The mechanism of respiratory control which would most likely depress the Q_{O_2} would be the lack of substrate within the mucosal cells. Since the cells have performed the increased work of transport the ATP/ADP ratio should favor increased respiration if adequate amounts of substrate are present. If then, substrate availability limits respiration, the decay of transport can be related to diminished ATP production. Moreover, since transport is sustained with glucose available to the bicarbonate-stimulated gallbladder, it would appear that the effect of the bicarbonate is to increase the utilization of both endogenous and exogenous substrates. Exogenous glucose is apparently more readily utilized in the bicarbonate effect since glucose added to bicarbonate-free preparations does not sustain fluid transport at initial levels.

According to the above argument it should be possible to stimulate fluid transport with added glucose in gallbladders which have had the energy

Table 3. Initial fluid transport rates and initial respiration rates for individual gallbladders unexposed to exogenous substrate

Exp.	Elapsed time (hr)	Transport (μliters/mg (dry wt) hr)	Q_{O_2} (μliters O_2/ mg (dry wt) hr)
0 mM HCO_3^-			
S_9	0–1	18.8	5.5
	4–5	5.6	6.6
	9–11	1.2	6.5
S_{10}	0–2	9.9	5.0
	3–4	4.2	6.9
S_{11}	0–2	17.7	7.6
	4–5	5.2	7.1
25 mM HCO_3^-			
S_{32}	0–1	53.5	16.6
	2–3	23.3	10.1
	6–7	16.7	25.6
S_{42}	0–1	12.3	9.4
	2–4	6.9	9.4
S_{43}	0–1	19.3	23.9
	4–5	8.9	20.6

Transport and respiration relative to initial rates are plotted in Fig. 2.

stores reduced by sustained metabolic activity. To establish these conditions transport rates were obtained for gallbladders immediately upon making the preparation and again after 20 to 24 hr at 4 °C. Table 4 shows the response of gallbladders which were placed overnight at 4 °C in the refrigerator in bicarbonate-free medium. After 24 hr they were rewarmed to 38 °C and transport again measured. The addition of bicarbonate ion brought about the characteristic increase in fluid transport. The data of Table 4 show the bicarbonate effect by alternate exposure of the gallbladder to bicarbonate-free Ringer's and then to bicarbonate Ringer's solution. The rates of transport are thus compared for eight different gallbladders. The second portion of Table 4 shows that the bicarbonate effect persists after the organ has been kept 20 to 24 hr at 4 °C. This second group of gallbladders was also exposed alternately to Ringer's solution containing 0 mM HCO_3 and 25 mM HCO_3.

As the overnight storage of gallbladders in the cold was successful the response of the transport system to exogenous glucose after overnight storage at 4 °C in bicarbonate Ringer's solution was then tested. This procedure would allow the bicarbonate ion to influence those metabolic pro-

Table 4. Effect of the bicarbonate ion on fluid transport in the *in vitro* rabbit gallbladder

Fluid transport (μliters/mg (dry wt) hr, $\bar{x} \pm$ SE (n))	
Rate of fluid transport for gallbladders alternately exposed to 0 mM HCO_3^- and 25 mM HCO_3^-	
0 mM HCO_3^-	12.3 ± 2.6 (8)
25 mM HCO_3^-	17.8 ± 2.4[a]
Rate of fluid transport for gallbladders alternately exposed to 0 mM HCO_3^- and 25 mM HCO_3^- after 20 to 24 hr at 4 °C	
0 mM HCO_3^-	10.7 ± 2.3 (12)
25 mM HCO_3^-	18.9 ± 2.0[b]

Fluid transport was measured on individual gallbladders alternately exposed to the bicarbonate ion and bicarbonate-free Ringer's solution in the absence of exogenous substrate.

[a] $p < 0.05$; 0 mM HCO_3^- *vs.* 25 mM HCO_3^-; paired variates.

[b] $p < 0.05$; 0 mM HCO_3^- *vs.* 25 mM HCO_3^-; unpaired variates. Transport measured at 38 °C.

Table 5. The effect of exogenous glucose added to gallbladders after storage at 4 °C (20 to 24 hr)

Fluid transport (μliters/mg (dry wt) hr, $\bar{x} \pm$ SE)	
Day 1, 12 gallbladders	
25 mM HCO_3^-, 0 mM glucose	11.6 ± 1.8
Day 2, 12 gallbladders of day 1, kept 20 to 24 hr at 4 °C	
25 mM HCO_3^-, 0 mM glucose	8.3 ± 1.2
0 mM HCO_3^-, 11 mM glucose	18.3 ± 2.7[a]

No significant difference for transport on day 1 compared to day 2 for no glucose with HCO_3^-.

[a] Transport on day 2, HCO_3^- *vs.* no HCO_3^- with glucose, highly significant. $p < 0.01$; unpaired variates. Transport measured at 38 °C.

cesses which affect transport at a reduced rate of energy utilization. The effect of the addition of glucose after this treatment was observed; that is, glucose was added in the absence of bicarbonate ion. There was no significant change in transport after the storage period in the absence of substrate. However, the addition of glucose in the absence of bicarbonate brings about a highly significant increase in the rate of transport (Table 5).

Table 6. The relationship between fluid transport and the concentration of the bicarbonate ion

Exp.	Bicarbonate (mM)	Fluid transport (μliters fluid/mg (dry wt) hr)	Ratio [a]	pH
S_{175}	0	11.3	1.00	7.2
	1.25	17.8	1.57	7.2
S_{176}	0	0.6	1.00	7.2
	1.25	3.6	6.03	7.2
S_{173}	0	19.3	1.00	7.2
	2.5	28.4	1.47	7.1
S_{174}	0	8.5	1.00	7.2
	2.5	18.5	2.16	7.4
S_{177}	0	19.0	1.00	7.2
	12.5	26.3	1.38	7.6

[a] Fluid transport with HCO_3^-/fluid transport without HCO_3^-.

This increase in transport was about equal to that observed for the addition of bicarbonate when added to gallbladders without substrate (Table 3). Therefore, the effect of the glucose addition may also be compared to the similar conditions expressed in Table 4 although these are different gallbladders. Exogenous glucose can therefore increase the basic rate of transport when it is added under appropriate conditions. Thus, the storage at 4 °C for 20 to 24 hr in bicarbonate Ringer's solution produces an energy-limiting circumstance for transport.

With respect to the bicarbonate effect an additional condition was assessed; i.e., the variation in transport rate with differing concentrations of bicarbonate ion. Table 6 presents data in which bicarbonate ion concentration is related to the bicarbonate ion effect upon fluid transport. None of the concentrations chosen produced changes which differ notably from those produced by 25 mM HCO_3 (*see also* Tables 2, 4 and 5). A possible exception is S_{176} in which the initial rate of transport without bicarbonate was very low. In any case, the range of concentrations used did not markedly affect transport. Shifts in extracellular pH produced by this procedure are slight and do not appear to influence the effect of the bicarbonate ion. However, these data do establish that the characteristic increase in transport occurred with the addition of the bicarbonate ion under conditions of slight pH shifts and low concentrations of bicarbonate.

Discussion

The observation of Diamond (1964) that the bicarbonate ion stimulated an increase in fluid transport in the gallbladder is confirmed. Diamond (1964) also showed that the reproducibility of transport measurements was within 10% standard deviation of the mean of the replications performed; the conditions of this reproducibility were bicarbonate ion in the Ringer's solution with added substrate. The data of Table 2 not only confirm the reproducibility established by Diamond (1964), but they very clearly demonstrate the necessity of both the bicarbonate ion and an exogenous substrate (glucose) to be present initially for the long term support of salt transport in the *in vitro* gallbladder.

A normal physiological function of the bicarbonate ion is revealed by the present experiments. Under normal physiological conditions the bicarbonate ion is present in mammalian blood at about 25 mM. Blood glucose would also be maintained at some relatively constant level. These circumstances then indicate that the gallbladder's absorptive function occurs *in vivo* at near optimum conditions at least with respect to these two variables.

The present data indicate that the function of the bicarbonate ion in the gallbladder mucosal cell is related to the normal utilization of energy sources. As a first indication of this function one observes that the lack of the bicarbonate ion and exogenous glucose brings about a decay of the transport function. Moreover, transport is minimal 4 hr after exposure to bicarbonate ion with no available exogenous energy source. Since respiration declines with time if bicarbonate ion only is present and exogenous glucose is absent, the conclusion is supported that the endogenous energy sources were utilized for the transport process. This idea is reinforced by the data showing a high rate of respiration in the absence of both bicarbonate and glucose. The respiratory level indicates that the energy stores were available and being utilized. However, the respiratory rate with respect to transport became less efficient; i.e., the available energy as ATP was apparently not channeled to salt transport. Thus, in the absence of bicarbonate the pace of substrate utilization continues at the expense of the transport function of the gallbladder mucosa.

Further evidence of this metabolic influence of the bicarbonate ion is provided by the response of gallbladders exposed to glucose after the gallbladder had been stored overnight at 4 °C in the presence of bicarbonate ion. This addition of glucose was made after transport had been measured in the rewarmed gallbladders which had been washed free of bicarbonate. The glucose then produced an increase in transport, an effect which had not been observed previously (Table 2). This interpretation is consistent with

and supports the previous conclusion that the bicarbonate ion affects the utilization of endogenous substrate.

The minimal concentration of bicarbonate ion required to produce the effect is less than 1.25 mM as this concentration produced the increases in transport. Thus, the critical concentration is likely to be less than 1 mM.

Carbon dioxide fixation may occur to provide an intermediate which functions in the maintenance of the energy supply for transport. One hypothesis which must be considered is the fixation of carbon dioxide to form tricarboxylic acid (TCA) cycle intermediates which can provide for increased respiratory activity (CO_2 fixation, *see* Greenberg, 1967). However, the operation of an anaplerotic path to replenish intermediates seems unlikely as adequate support of transport by acetate (Table 2) shows there must be sufficient TCA cycle intermediates to metabolize the exogenous acetate. Moreover, the level of respiration under bicarbonate declines with time, an observation which is inconsistent with a state of accelerated turnover of the TCA cycle. An alternative hypothesis involving the fixation of carbon dioxide is considered in a companion manuscript (Martin & Murphy, 1974).

Previously, carbon dioxide effects on epithelial sodium transport systems of the frog skin had been noted by Martin (1962). Funder, Ussing and Wieth (1967) showed that bicarbonate protects against the decline in extracellular pH or it positively stimulates short-circuit current. In the turtle urinary bladder, sodium transport is positively stimulated by bicarbonate ion in the serosal medium and the effect requires glucose to be evident; in addition, the effect can be elicited under anaerobic conditions (Gonzales, Shamoo & Broadsky, 1969). Metabolic support of the isolated toad urinary bladder has been investigated by Maffley and Edelman (1963) where specific substrate interactions with sodium transport were demonstrated. The synergistic effects of substrates on aldosterone-induced increases in transport have been investigated by Fimognari, Porter and Edelman (1967). However, the relationship between bicarbonate and metabolism in these preparations apparently remains to be investigated.

Bicarbonate ion is almost always present in cells and tissues and for this reason its apparent involvement in the shift of metabolic events is obscured. However, the present evidence indicates that the bicarbonate ion is involved in the channeling of energy for specific cellular requirements such as transport.

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