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THE ACCELERATING EFFECT OF SEROSAL HCO_3^- ON Na^+ TRANSPORT IN SHORT-CIRCUITED TURTLE BLADDERS

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SUMMARY

The isohydric addition of HCO_3^- to the serosal fluid (final concn., 17 mM) of short-circuited turtle bladders produces a sustained increase (near-doubling) in the rate of Na^+ transport from mucosa to serosa. A qualitatively similar, but smaller, effect was obtained from addition of Cl^- to the serosal fluid. The anion-induced acceleration of Na^+ transport was independent of the presence or absence of HCO_3^- and/or Cl^- in the mucosal fluid. Whereas the transport-accelerating effect of HCO_3^- could be superimposed upon that of Cl^-, the reverse did not hold. The presence and utilization of glucose is apparently an absolute requirement for eliciting the HCO_3^- effect on Na^+ transport. On the other hand, the effect was elicited in anoxic as well as in oxygenated bladders. The O_2-induced recovery of Na^+ transport from N_2-anoxia is dependent upon the presence of serosal HCO_3^-.

INTRODUCTION

The isolated bladder of the fresh water turtle (*Pseudemys scripta*) actively transports Na^+, Cl^- and HCO_3^- from the mucosal (m) to the serosal (s) bathing fluid. The active transport of Na^+ has been demonstrated under two independent sets of conditions: *i.e.*, in open-circuit bladders mounted in the form of sacs and in short-circuited bladders mounted in the form of membranes and bathed on both surfaces by Na^+-rich Ringer solutions containing HCO_3^- (refs. 1–3). KLAHR AND BRICKER[4] and recently STEINMETZ *et al.*[5], under still another set of conditions, demonstrated active Na^+ transport across short-circuited bladders bathed in HCO_3^--free Ringer solutions.

The active transport (m to s) of Cl^- has also been demonstrated under two independent sets of conditions, *i.e.*, by chemically measuring Cl^- concentration changes in sac preparations bathed by HCO_3^--rich Na^+-free Ringer solutions[2] and by measuring[36] Cl^- fluxes in short-circuited preparations bathed by HCO_3^--rich Ringer solutions with and without Na^+ (ref. 6).

There may or may not be net Cl^- transport across shortcircuited bladders bathed in HCO_3^--free Ringer solutions under which conditions I_{Na+} was approximately equal to I_{sc} (refs. 4, 5 and 7) However, no measurements of Cl^- flux, chemical or isotopic, have been reported in the HCO_3^--free systems.

The active transport of HCO_3^- (m to s), rather than that of H^+ (s to m), has been established directly on the basis of finding simultaneous decreases in pH, HCO_3^- concentration and pCO_2 in the mucosal fluid of sacs bathed by Na^+-Ringer solution containing HCO_3^- (ref. 8). This has been discussed in detail elsewhere[9]. The possibility of H^+ transport from s to m, especially in bladders bathed by HCO_3^--free Ringer solutions, has been invoked by STEINMETZ[10] in order to account for the observed acidification of the HCO_3^--free mucosal fluid.

Previous data from this laboratory have demonstrated that under short-circuiting conditions, net Na^+ flux is independent of the presence of Cl^- and HCO_3^- in the mucosal fluids[3]. However, data presented previously in abstract form[11,12] have suggested that net Na^+ flux does vary as a function of changes in the anionic composition of the serosal fluid.

The purposes of the present study, a follow-up of the aforementioned preliminary data, are as follows: (1) to establish the nature and extent of the effect of serosal HCO_3^- (and/or Cl^-) on Na^+ transport; (2) to compare the effects of HCO_3^- (and/or Cl^-) acting on the serosal surface with those of the same anions acting on the mucosal surface; (3) to determine the O_2 and glucose dependency of such anionic effects.

METHODS

Urinary bladders of the turtle (*P. scripta*) were excised and mounted in a modified Ussing chamber. The techniques for excision and mounting, the structure of the lucite chamber and the impelled circulation of ambient fluids have been described previously[6].

Paired aliquots of the same bladder were mounted simultaneously in the double-barreled Lucite chamber and were short-circuited. Electrical measurements, made by techniques described previously[6], included short-circuiting current (I_{sc}), instantaneous transbladder potential difference (PD) and transbladder resistance (R).

Techniques for adding isotopes (^{24}Na and ^{22}Na) and for estimating transbladder fluxes have been described in detail previously[3].

The compositions of the bathing solutions, in terms of final mM concentrations, were as follows: (a) HCO_3^--rich Na^+ Ringer: Na^+, 101; Cl^-, 92; K^+, 4.8; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; HCO_3^-, 17; Mg^{2+}, 0.8; SO_4^{2-}, 0.8; and glucose, 11. Final osmolality 220 mosmoles. (b) NaCl Ringer: Na^+, 101; Cl^-, 92; K^+, 4.8; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; monomethyl sulfate, 17; Mg^{2+}, 0.8; SO_4^{2-}, 0.8; and glucose, 11. Final osmolality 220 mosmoles. (c) Sodium methyl sulfate Ringer: Na^+, 101; monomethyl sulfate, 109; K^+, 4.8; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; Mg^{2+}, 0.8; SO_4^{2-}, 0.8; and glucose, 11. Final osmolality 220 mosmoles. (d) HCO_3^--rich sodium methyl sulfate Ringer: Na^+, 101; monomethyl sulfate, 92; K^+, 4.8; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; HCO_3^-, 17; Mg^{2+}, 0.8; SO_4^{2-}, 0.8; and glucose, 11. Final osmolality 220 mosmoles. (e) HCO_3^- solution: Na^+, 105; K^+, 5.0; HCO_3^-, 110.

Chemical measurements of Na^+, Cl^-, K^+ and HCO_3^- concentration and pH and osmolality were performed routinely on all ambient fluids by techniques described previously[13,3]. In each experiment, fluids were analyzed before and after incubation with the bladder.

When in contact with the bladder, the measured pH of Na^+-Ringer solutions containing HCO_3^- was kept between 7.4 and 7.6 pH units by continuous gassing

with O_2–CO_2 (99:1, v/v) and that of the HCO_3^- free Na^+ Ringer solutions was fixed by continuous gassing with 100 % O_2.

The area of exposed bladder wall across which ion fluxes occur (and with respect to which all measured fluxes refer) was 1.5 cm^2, and the dried weight of this area of exposed tissue was 14–18 mg.

All experiments were performed at 22–25°.

The design of the experiments was to add isohydrically 1.5 ml of 110 mM $NaHCO_3$ to the serosal fluid (12 ml) of one of a pair of mounted half-bladders. This maneuver assured that the serosal surface of the bladder was bathed by a solution containing HCO_3^- at a concentration of approx. 17 mM. Alternatively, the same results were obtained when the entire 12 ml of HCO_3^--free serosal fluid were removed and replaced by 12 ml of a HCO_3^--containing Ringer solution. The mated half-bladder was used as a control for the effect of aging during the course of the experiments.

The terms "isohydric addition of HCO_3^-" to the serosal fluid refers to the addition of HCO_3^- (final concn. 17 mM) simultaneously with the addition of CO_2 (1–3 %) to the gas mixture. The partial pressure of CO_2 in O_2 was predetermined as that required to maintain constancy of the pH of the bathing solution previously gassed with 100 % O_2. In short, concomitant with addition of HCO_3^- to the Ringer solution, the gas mixture was changed from 100 % O_2 to O_2– CO_2 (99:1, v/v) in order to maintain a constant pH of 7.5 in the ambient fluids.

In experiments in which Cl^- was added to the serosal fluid, the entire 12 ml of Cl^--free serosal fluid were removed and replaced by 12 ml of a Ringer solution containing 91.5 mM Cl^-.

RESULTS

Effect of serosal HCO_3^- on Na^+ transport

In a preliminary series of four experiments on paired half-bladders, one half-bladder of each pair was bathed on both surfaces, mucosal and serosal, by Na^+-Ringer solution containing 17 mM HCO_3^- and gassed with O_2–CO_2 (99:1, v/v) while its mated half was bathed on both surfaces by Na^+-Ringer solution without HCO_3^- and gassed with 100 % O_2. Net Na^+ current, I_{Na^+}, was determined from the simultaneously occurring unidirectional fluxes of $^{22}Na^+$ and $^{24}Na^+$.

TABLE I

EFFECT OF AMBIENT HCO_3^- ON Na^+ TRANSPORT

Mean values and range of values of I_{Na^+}, I_{sc} and PD in four pairs of hemibladders, in which each half-bladder was bathed in HCO_3^--rich Ringer solution while its mated half was bathed in HCO_3^--free Ringer solution.

$[HCO_3^-]$ (mM)	I_{Na^+} (μA)	I_{sc} (μA)	PD (mV)
17	191 (142–237)	131 (114–161)	49 (36–61)
0.2	117 (72–150)	110 (65–155)	55 (33–69)

Table I presents mean values and ranges of values for I_{Na+}, I_{sc} and transbladder potential (PD) in the four mated pairs of half-bladders.

The mean values of I_{Na+} and I_{sc} across each hemibladder in HCO_3^--containing Ringer solution were on the average 58 and 18 % greater respectively, than the corresponding values across each of the mated hemibladders in HCO_3^--free Ringer solution. Whereas, I_{Na+} exceeded I_{sc} by 60 μA in the HCO_3^--enriched system, I_{Na+} did not differ significantly from I_{sc} in the HCO_3^--free system.

The excess of I_{Na+} over I_{sc} in bladders bathed by HCO_3^--containing Ringer solution confirms data of our previous reports on these current densities in the short-circuited bladder[3]. In contrast, the approximate equality between I_{Na+} and I_{sc} in bladders bathed by HCO_3^--free Ringer solution is consistent with values reported by other workers using similar HCO_3^--free bathing fluids on the bladders[4,5,7].

The new finding in the data of Table I is that Na^+ transport is apparently dependent upon the presence of HCO_3^- on both the serosal and mucosal surfaces. Together with our previous demonstration of the independence between Na^+ transport and the presence of HCO_3^- on the mucosal surface[3], the data in Table I suggest that the effect of HCO_3^- on I_{Na+} is due to the presence of the anion on the serosal but not on the mucosal surface.

To establish that the HCO_3^- effect was elicited from the serosal surface, but not from the mucosal surface, two sets of experiments were designed as follows. In the first set, paired aliquots of a single bladder were bathed initially on both surfaces by identical HCO_3^--free Cl^--free Ringer solutions. HCO_3^- was added isohydrically to the serosal fluid of one of the mated half-bladders but not to that of the mated half-bladder which served as a control for aging effects. In the second set, the same

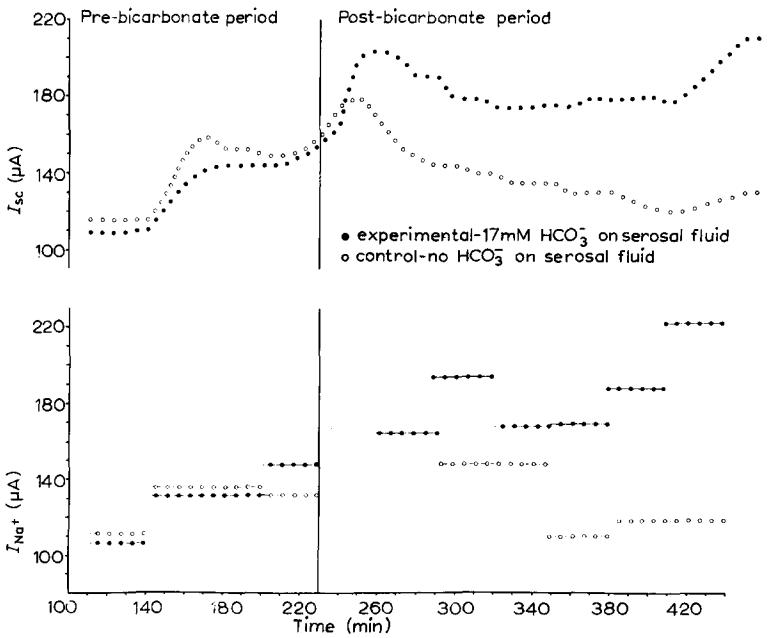

Fig. 1. I_{sc} and I_{Na^+} *vs.* concurrent time in a mated pair of hemibladders before and after addition of HCO_3^- to the serosal fluid of one member of the pair (●), but not to that of the other member (○).

maneuver (addition of HCO_3^- to the serosal fluid) was performed on one of a pair of mated half-bladders, bathed initially with HCO_3^--free Ringer on the serosal surface, but bathed throughout the experiment with HCO_3^--containing Ringer solution on the mucosal surface.

Fig. 1, showing data from one of the first set of experiments alluded to, is a plot of values of I_{sc} and of I_{Na^+} *versus* those of concurrent time in a pair of half-bladders from a single piece of tissue. The initial bathing fluids (serosal and mucosal) were identical sodium methyl sulfate Ringer solutions gassed with 100 % O_2.

After 90 min of incubation in the chamber, simultaneously occurring unidirectional fluxes of $^{22}Na^+$ and $^{24}Na^+$ were determined for three to four periods, each of 30–40 min duration. At 230 min, 1.5 ml of HCO_3^- solution were added isohydrically (see composition of added fluid in METHODS) to the serosal fluid of one of the paired half-bladders, and a similar volume of a HCO_3^--free solution was added to the control half.

It can be seen that addition of HCO_3^- to a final concentration of 17 mM in the serosal fluid induced increases in I_{sc} and I_{Na^+} that lasted until the end of the experiment. The HCO_3^--induced increase of I_{Na^+} lagged behind but paralleled that of I_{sc}. Such changes were not present in the transport parameters of the control half-bladder, which continued to decay with time. Similar chnges were observed in six other experiments performed under the same conditions as those described for the experiment depicted in Fig. 1. Results obtained from these experiments are shown in Table II.

Table II presents average values in each of seven experiments for I_{Na^+}, I_{sc} and PD before and after the addition of HCO_3^- to the serosal fluid of the experimental half-bladders and before and after the addition of a similar volume of HCO_3^--free Ringer solution to the serosal fluid of the control half-bladders. The terms before and after, as used in the table, refer to the flux periods beginning 2 h before the addition of HCO_3^- and to those flux periods beginning 40–60 min after the addition of HCO_3^-, respectively.

In five experiments, I_{Na^+} and I_{sc} of the experimental half-bladders increased after the addition of HCO_3^-, while the same parameters of the paired controls remained constant or decreased slightly. In the two instances (Expts. IV and VI) where levels of I_{Na^+} and I_{sc} of both experimental and control bladders decreased, the rate of decrease in the HCO_3^--enriched half-bladder was much slower than that in the paired HCO_3^--free half-bladder.

The grand means for all seven pairs of parameters in both HCO_3^--enriched and paired control half-bladders are shown in the last two rows of the upper portion of the table. After addition of HCO_3^- to the serosal fluid, the mean value for I_{Na^+} increased from 140–166 μA, an acceleration which is retarded to some extent by the simultaneous occurrence of time-dependent decreases in I_{Na^+} and in the other parameters. The extent of such time-dependent changes may be estimated from the concomitantly obtained data in the paired control half bladders; *e.g.*, the mean value for I_{Na^+} in all seven paired controls decreased from 121–91 μA as a function of time. The data on grand means indicate that HCO_3^--induced increases occur concomitantly with time-dependent decreases in I_{Na^+} and I_{sc}. At the same time, the addition of HCO_3^- retards the time-dependent decay of PD and accelerates the time-dependent decrease in resistance (not shown in Table II).

TABLE II

EFFECT OF SEROSAL HCO_3^- ON Na^+ TRANSPORT PARAMETERS IN THE ABSENCE OF AMBIENT Cl^-

Average values of I_{Na+}, I_{sc} and PD, before and after addition of HCO_3^- to the serosal fluid of seven hemibladders denoted as experimental compared to those before and after a dummy addition to the mated hemibladder denoted as Control. Grand means and statistical parameters for all seven pairs are shown in the lower portions of the table. The difference in the average value of each parameter before and after addition of HCO_3^- was obtained for experimental and control half-bladder. These differences (after *minus* before) in each experimental half (*e.g.* ΔI_{Na^+}, Experimental) less those in each paired control half (*e.g.*, ΔI_{Na^+}, Control) were designated $\Delta(\Delta I_{Na^+})$, $\Delta(\Delta I_{sc})$, and $\Delta(\Delta PD)$ — thus providing statistically analyzable data corrected for the time-dependent effects.

Expt. No.	*Period*	I_{Na^+} (μA)		I_{sc} (μA)		*PD (mV)*	
		Experimental	*Control*	*Experimental*	*Control*	*Experimental*	*Control*
I	Before	112	128	108	135	84	81
	After	116	60	96	62	48	20
II	Before	28	46	33	55	30	37
	After	59	51	78	66	50	34
III	Before	141	87	146	74	58	19
	After	226	81	208	80	45	17
IV	Before	97	114	91	96	73	68
	After	77	56	67	36	39	21
V	Before	130	127	134	143	102	97
	After	187	128	185	135	76	73
VI	Before	279	247	244	240	95	95
	After	246	140	242	127	85	67
VII	Before	192	97	205	90	89	48
	After	250	121	233	107	77	43
Grand means	Before	140	121	137	119	75.9	63.6
	After	166	91.0	158	87.6	60.0	39.3
		Δ (Δ I_{Na^+})		Δ (Δ I_{sc})		Δ (ΔPD)	
Mean ± S.E.		56.2 ± 9.0		52.6 ± 11.8		8.4 ± 5.6	
Probability		$P < 0.001$		$P < 0.001$		$0.1 < P < 0.2$	

The HCO_3^--induced changes in transport parameters of the half-bladders were compared statistically with the concomitant time-dependent changes in the mated half-bladders. It can be seen that the HCO_3^--induced changes in I_{Na^+} and I_{sc}, $\Delta(\Delta I_{Na^+})$ and $\Delta(\Delta I_{sc})$, were approximately equal to each other and significantly different from zero ($P > 0.001$).

Although the decreases in PD of the experimental halves appeared to be less than those of the mated controls, this could not be substantiated on statistical grounds, *e.g.*, for $\Delta(\Delta PD)$ and $P > 0.1$).

Not shown is a statistically significant effect of serosal HCO_3^- on the transmural resistance (R). The mean value and standard error for the HCO_3^--induced change in R, $\Delta(\Delta R)$, of the seven bladder pairs was 100 ± 33 Ω ($P < 0.05$).

In addition to the effect of serosal HCO_3^-, the data of the table provide pertinent information on the conditions required for matching of I_{Na^+} with I_{sc} in the turtle blad-

der. In effect, I_{Na+} approximated I_{sc} in each and every experimental and control bladder. The close approximation of I_{Na+} and I_{sc} confirmed our previous data obtained with sodium methyl sulfate on the mucosal surface together with HCO_3^--enriched Na^+ Ringer solution on the serosal surface[3] as well as confirming those of STEINMETZ *et al.*[5] obtained with Na_2SO_4 Ringer solution on both mucosal and serosal surfaces.

Not shown are the levels of unidirectional back flux (s to m) of Na^+ which increased with time in both the HCO_3^--treated and paired control half-bladders. During the periods after HCO_3^- addition, the mean value and standard error of back flux in the experimental half-bladders (5.08 ± 2.63) was statistically the same as that (5.33 ± 1.62) in the paired controls ($P > 0.9$). In other words, HCO_3^- addition had no detectable effect on the back flux of Na^+. Therefore, the HCO_3^--induced acceleration of net Na^+ flux can be estimated with reasonable accuracy from measurements of the increases in forward (m to s) flux. Accordingly, further data presented herein will include measurements of forward (m to s) I_{Na+} and I_{sc}.

The purpose of the next set of experiments was to determine whether the HCO_3^--induced acceleration of I_{Na+} could be elicited in the presence of Cl^--rich as well as it had been in the presence of Cl^--free (methyl sulfate) Ringer solutions.

Table III presents mean values for $I_{Na^+}^{ms}$, I_{sc} and PD obtained from five experiments in which bladders were bathed initially by NaCl-Ringer solutions. This Ringer solution was identical to that used in the experiments shown in Fig. 1 and Table II, except that NaCl was substituted for sodium methyl sulfate.

After addition of HCO_3^- to the serosal fluid of the experimental half-bladder, the m to s flux of Na^+ increased relative to the concomitant changes in paired control bladders which were subjected to a dummy addition of HCO_3^--free Ringer solution.

The HCO_3^--induced increments of forward I_{Na+} and I_{sc}, paralleling one another, were significantly different from the concomitant time-dependent changes in the paired controls. Moreover, the magnitude of the increment in I_{Na+}^{ms} was statistically the same as that in the I_{sc}.

TABLE III

EFFECT OF SEROSAL HCO_3^- AND Na^+ TRANSPORT PARAMETERS IN THE PRESENCE OF AMBIENT Cl^-

Mean values for $I_{Na^+}^{ms}$, I_{sc} and PD in five paired hemibladders before and after addition of HCO_3^- to the serosal fluid of the experimental, but not to that of the control hemibladder. Lower portion of table presents mean values, S.E., and probability estimates for Δ (ΔI_{Na^+}), Δ (ΔI_{sc}), and Δ (Δ PD) — which are defined in the legend to Table II.

	Period	$I_{Na^+}^{ms}(\mu A)$		I_{sc} (μA)		*PD (mV)*	
		Experimental	*Control*	*Experimental*	*Control*	*Experimental*	*Control*
Mean values ($n = 5$)	Before	115	111	95.0	95.2	59.0	64.4
	After	161	117	146	106	60.2	59.2
		Δ (ΔI_{Na^+})		Δ (ΔI_{sc})		Δ (Δ PD)	
Mean ± S.E.		39.9 ± 4.91		39.8 ± 6.74		7.2 ± 1.57	
Probability		$P < 0.001$		$0.001 < P < 0.01$		$0.01 < P < 0.02$	

In summary, the data in Fig. 1 and in Tables II and III show that HCO_3^-, added isohydrically to the serosal fluid but not to the mucosal fluid, induces a significant acceleration in the net flux and forward flux of Na^+ as well as a decrease in the d.c. resistance of the turtle bladder *in vitro*.

The purpose of the next set of experiments was to determine whether the serosally oriented, anion-induced acceleration of I_{Na^+} could be elicited in the presence of HCO_3^- on the mucosal surface. Such an acceleration did occur as is shown in the following table.

Table IV, similar in format to Table II, presents mean values for I_{Na^+}, I_{sc} and PD in five experiments. Initially, the mucosal surface of both experimental and control bladders was bathed by NaCl-Ringer solution containing 17 mM HCO_3^-, while the serosal surface was bathed by HCO_3^--free NaCl Ringer solution. After three to four control periods, HCO_3^- was added to the serosal fluid of the experimental half, and a similar volume of a HCO_3^--free Ringer was added to the control half-bladder.

TABLE IV

EFFECT OF SEROSAL HCO_3^- ON Na^+ TRANSPORT PARAMETERS IN THE PRESENCE OF MUCOSAL HCO_3^-
Mean values for $I^{ms}_{Na^+}$, I_{sc}, and PD in five paired hemibladders before and after addition of HCO_3^- to the serosal fluid of the experimental, but not to that of the control hemibladder. Lower portion of table presents mean values, S.E., and probability estimates for Δ (ΔI_{Na^+}), and Δ (ΔI_{sc}), Δ (Δ PD) — which are defined in the legend to Table II.

	Period	$I^{ms}_{Na^+}$ (μA)		I_{sc} (μA)		*PD* (*mV*)	
		Experimental	*Control*	*Experimental*	*Control*	*Experimental*	*Control*
Mean values ($n = 5$)	Before	142	123	114	115	74.8	66.8
	After	204	119	168	119	78.8	59.6
		Δ (ΔI_{Na^+})		Δ (ΔI_{sc})		Δ (Δ PD)	
Mean ± S.E.		62.4 ± 12.7		47.6 ± 6.83		6.40 ± 3.78	
Probability		$0.001 < P < 0.01$		$P < 0.001$		$0.1 < P < 0.2$	

Addition of HCO_3^- to the serosal fluid of the experimental halves induced a reproducible and significant acceleration of $I^{ms}_{Na^+}$ and I_{sc}. This acceleration was qualitatively and quantitatively similar to that described in Tables II and III. Thus, the effect of serosal HCO_3^- on Na^+ transport is the same in the presence as it is in the absence of HCO_3^- on the mucosal surface. In addition, the flux and electrical characteristics of control bladders bathed for 300 min by a HCO_3^--containing mucosal fluid were essentially the same as those of control bladders bathed for the same time by a HCO_3^--free mucosal fluid (*cf.* Table IV with Tables II and III).

These data demonstrate that the transport-accelerating action of HCO_3^- is elicited from the serosal but not from the mucosal surface. As long as HCO_3^-, at these concentrations, bathes the mucosal fluid, HCO_3^- will be transported from mu-

cosa to serosa[8]. Ultimately, such a transport of HCO_3^- would deliver enough HCO_3^- into the serosal fluid to trigger the HCO_3^--sensitive acceleration of Na^+ transport. Whereas theoretically possible, such a "spontaneous" stimulation of cation transfer did not occur in the 300 min allowed in the experiments shown in Table IV.

Effect of serosal Cl^- on Na^+ transport

If the HCO_3^--induced acceleration of I_{Na+} is due to the anionic nature of HCO_3^-, then other anions (*e.g.*, Cl^-) manipulated in the same way would produce a qualitatively similar effect. We expected that Cl^- added to the serosal, but not to the mucosal, fluid would increase Na^+ transport on the basis of two observations considered together: (a) Our own demonstration that Na^+ transport was not affected by mucosal Cl^- (ref. 3) and (b) that of STEINMETZ *et al.*[5] showing that Na^+ transport in the presence of Cl^- in both mucosal and serosal fluids was greater than that found in the absence of ambient Cl^-.

Therefore, the next set of experiments was designed and carried out in a manner identical to those involving serosal HCO_3^-, except that Cl^- rather than HCO_3^-, was added to serosal fluid. Results are presented in Tables V and VI.

TABLE V

EFFECT OF SEROSAL Cl^- ON Na^+ TRANSPORT PARAMETERS IN THE ABSENCE OF AMBIENT HCO_3^-

Mean values for $I_{Na^+}^{ms}$, I_{sc}, and PD in five paired hemibladders before and after addition of Cl^- to the serosal fluid of the experimental, but not to that of the control hemibladder. Lower portion of table presents mean values, S.E., and probability estimates for Δ (ΔI_{Na^+}), Δ (ΔI_{sc}), and Δ (Δ PD) — which are defined in the legend of Table II.

	Period	$I_{Na^+}^{ms}$ (μA)		I_{sc} (μA)		*PD* (*mV*)	
		Experimental	*Control*	*Experimental*	*Control*	*Experimental*	*Control*
Mean Values ($n = 6$)	Before	157	164	150	155	85.2	87.0
	After	173	155	161	139	64.3	61.7
		Δ (ΔI_{Na^+})		Δ (ΔI_{sc})		Δ (Δ PD)	
Mean ± S.E.		26.3 ± 10.0		27.8 ± 8.1		4.83 ± 3.8	
Probability		$0.02 < P < 0.05$		$0.01 < P < 0.02$		$0.2 < P < 0.3$	

Table V, similar in format to Table IV, shows mean values for $I_{Na^+}^{ms}$, I_{sc} and PD in six experiments before and after the addition of Cl^- to the serosal fluid of the experimental but not to that of the control half-bladder. Both half-bladders were bathed initially by sodium methyl sulfate Ringer solution without HCO_3^- and without Cl^-. The addition of serosal Cl^- was accomplished by substitution of NaCl-Ringer for sodium methyl sulfate Ringer solution.

After addition of Cl^-, the mean values of $I_{Na^+}^{ms}$ and I_{sc} increased significantly in the experimental half-bladders. During the same time, mean values of those parameters decreased slightly in the control half-bladders. The statistical analysis

TABLE VI

EFFECT OF SEROSAL Cl^- ON Na^+ TRANSPORT PARAMETERS IN THE PRESENCE OF AMBIENT HCO_3^-

See legend to Table V.

	Period	$I_{Na^+}^{ms}$ (μA)		I_{sc} (μA)		*PD* (*mV*)	
		Experimental	*Control*	*Experimental*	*Control*	*Experimental*	*Control*
Mean values ($n = 5$)	Before	220	224	213	224	89.6	86.6
	After	216	206	190	192	58.8	79.6
		Δ (ΔI_{Na^+})		Δ (ΔI_{sc})		Δ (ΔPD)	
Mean ± S.E.		13.8 ± 17.2		8.60 ± 24.9		23.8 ± 19.0	
Probability		$0.4 < P < 0.5$		$0.7 < P < 0.8$		$0.2 < P < 0.3$	

shown in the table (analogous to the analysis applied to data of previous tables) showed that the Cl^--induced increments of flux and current, corrected for aging effects, were statistically significant.

Table VI, similar in format to the previous tables, shows mean values for $I_{Na^+}^{ms}$, I_{sc} and PD when Cl^- was added to bladders bathed in sodium methyl sulfate Ringer solution containing 17 mM HCO_3^-. No significant change in $I_{Na^+}^{ms}$ and I_{sc} was elicited. The only change was a slight decrease in both parameters, probably due to aging.

A comparison of the mean values of $I_{Na^+}^{ms}$ and I_{sc} in Table VI against the corresponding values in Table V shows that the magnitude of both parameters in Table VI are larger than those in Table V. This confirms what was shown before, namely that HCO_3^- enhances Na^+ transport.

The stimulating effect of Cl^- upon Na^+ transport, suggested by the results of Table V, is not present in Table VI. It is concluded that there is no superimposable effect of Cl^- on the HCO_3^- enhancement of Na^+ transport. On the other hand, the HCO_3^- effect on Na^+ is superimposable upon that of Cl^- (see Table III).

Metabolic dependency of the HCO_3^- effect

The effect of serosal HCO_3^- on Na^+ transport could have been due to interaction with the pump mechanism and/or with some components of the metabolic system supplying free energy for the pump. Therefore, experiments were designed to determine the dependence of the HCO_3^- effect upon the availability of exogenous O_2 and glucose.

HCO_3^- effect on Na^+ transport under conditions of N_2-induced anoxia. Paired half-bladders, mounted in the double-barreled lucite chamber, were bathed with sodium methyl sulfate Ringer solutions on both the mucosal and serosal surfaces. In the experimental halves, the mucosal fluid was HCO_3^--free sodium methyl sulfate Ringer solution gassed with 100% N_2, and the serosal fluid was sodium methyl sulfate Ringer solution containing 17 mM HCO_3^- gassed with N_2–CO_2 (99:1, v/v). In the paired control halves, both mucosal and serosal fluids were HCO_3^--free sodium methyl sulfate Ringer solutions gassed with 100% N_2. The pH of all bathing fluids remained fixed between 7.4 and 7.6 during all experiments.

TABLE VII

EFFECT OF THE PRESENCE OF SEROSAL HCO_3^- DURING N_2-ANOXIA

Mean values and statistical parameters for $I^{ms}_{Na^+}$, I_{sc}, PD and R in five mated pairs of half-bladders. In the row designated, 17 mM, mucosal fluid was HCO_3^--free sodium methyl sulfate Ringer solution, gassed with 100% N_2, while serosal fluid was sodium methyl sulfate Ringer solution with 17 mM HCO_3^-, gassed with N_2–CO_2 (99:1, v/v). In the row designated, 0.2 mM, both mucosal and serosal fluid were HCO_3^--free sodium methyl sulfate Ringer solution gassed with 100% N_2.

	Serosal $[HCO_3^-]$ *(mM)*	$I^{ms}_{Na^+}$ *(μA)*	I_{sc} *(μA)*	*PD (mV)*	*R (Ω)*
Mean	17	44.8	33.7	26	736
S.E.		3.9	3.2	3.4	72
Mean	0.2	17.9	8.3	12.6	1827
S.E.		5.1	1.6	2.4	209
Δ Mean*		26.9	25.4	13.4	1091
Probability*		$P < 0.001$	$P < 0.001$	$P < 0.02$	$P < 0.001$

* Mean differences of parameters in HCO_3^--rich and HCO_3^--free systems with probability that such differences were due to chance.

Table VII presents mean values for $I^{ms}_{Na^+}$, I_{sc}, PD and R in five pairs of mated half-bladders under the conditions described above and partly shown in the column pertaining to serosal HCO_3^-. No additions to or substitutions for bathing fluids were made in this set of experiments.

The mean levels of $I^{ms}_{Na^+}$, I_{sc} and PD reached under conditions of nitrogenation were approx. 1/4th to 1/5th of the corresponding mean levels reached under conditions of oxygenation (*cf.* data in Table VII with those in Table II). It should be noted that the mean levels of I_{Na^+}, I_{sc} and PD are those for the incubation period between 100 and 300 min in each experiment. Although the mean levels suggest the presence of steady-state levels, there were gradual decreases in the consecutive levels of I_{Na^+} and I_{sc} during the period of incubation, meaning that the final levels reached after 250–300 min were somewhat less than the mean values for 100–300 min. In the case of the "HCO_3^--free" bladders, the consecutive levels of I_{Na^+}, I_{sc} and PD decreased gradually and approached zero after 150–200 min of incubation. In the case of the HCO_3^--enriched bladders, the consecutive levels of I_{Na^+}, I_{sc} and PD decreased slightly and approached a finite level (5–10 μA less than the mean shown in the table) after 150–200 min of incubation.

However, the pertinent point about the data obtained during nitrogenation was that the HCO_3^--induced acceleration of Na^+ transport was clearly elicited. The mean values of $I^{ms}_{Na^+}$ and I_{sc} (44.8 and 33.7 μA) in the presence of serosal HCO_3^- were 2.5 and 4 times greater than the corresponding values obtained from paired controls in HCO_3^--free bathing fluids. In addition, the mean level of PD in the presence of serosal HCO_3^- was twice that found in the absence of HCO_3^-.

With serosal HCO_3^-, R during anoxia was 2 times the corresponding value found during oxygenation; and without serosal HCO_3^-, R during anoxia was 3 times the value found during oxygenation. The effect of HCO_3^- itself on R during anoxia was

of special interest. Data in the table show that the mean value for resistance in the presence of serosal HCO_3^-, 765 Ω, was less than 40 % of that (2034 Ω) in the absence of HCO_3^-.

The purpose of the next set of experiments was to determine whether the recovery of Na^+ transport, in going from nitrogenation to oxygenation of the media, was dependent upon the presence of serosal HCO_3^-.

Fig. 2, a plot of values of I_{sc} *versus* time, shows the pattern of recovery from anoxia after introduction of O_2 to the bathing fluids in one of two experiments on paired half-bladders. One half-bladder was bathed by HCO_3^--containing serosal fluid, while its mated half was bathed by HCO_3^--free fluids.

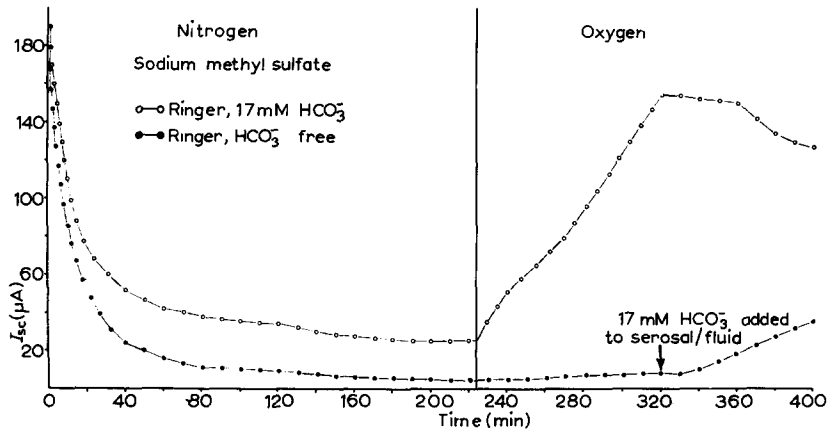

Fig. 2. HCO_3^- dependence of O_2-induced restoration of transport. I_{sc} *vs.* time in a mated pair of half-bladders incubated initially in nitrogenated fluids. ○, data from the half-bladder bathed by HCO_3^--enriched serosal fluid; ●, data from the mated half bathed HCO_3^--free media.

The introduction of N_2 into the bathing fluids was followed within 5 min by abrupt decreases of I_{sc} in both the HCO_3^--rich and HCO_3^--free systems. After 80 min of nitrogenation, the I_{sc} of both preparations reached steady-state levels with that of the HCO_3^--rich system being clearly greater than that of the HCO_3^--free preparation.

At 224 min, bubbling of O_2 was substituted for that of N_2 in all bathing fluids. In the HCO_3^--enriched system, I_{sc} increased sharply within a minute of introduction of O_2 and reached values of 130 μA in approx. 90 min. No such increase occurred after introduction of O_2 into the HCO_3^- free bladder system.

At 320 min, HCO_3^- was added to the serosal fluid of the mated control (*i.e.*, to the HCO_3^--free half-bladder) after which the level of I_{sc} increased steadily and began to approach that of the half-bladder which had been bathed by serosal HCO_3^- throughout the experiment.

Apparently the O_2-induced recovery from anoxia depends upon the presence of serosal HCO_3^-.

All the experiments shown heretofore were performed with glucose, at a concentration of 11 mM, in the ambient fluids. This raised the question, mentioned previously, concerning the glucose (or substrate) dependency of the HCO_3^- effect.

Dependency of HCO_3^- effect on glucose. Whereas removal of exogenous glucose

is a straightforward procedure, removal of endogenous glucose depends upon depletion of the glycogen stores of the epithelial cells. It has been shown that the glycogen content of isolated bladders decreases as a function of time of incubation in glucose-free Ringer solution and that this decrease is accompanied by a decrease in the rate of Na^+ transport[14].

In our hands, incubation of short-circuited bladders in substrate-free Ringer solution was accompanied by decreases in I_{sc} which reached one-third or less of the initial levels after 2–4 h of incubation.

Fig. 3 is a plot of I_{sc} *versus* time in a pair of half-bladders, both bathed by glucose-free HCO_3^--free oxygenated Ringer solution. At 130 min, HCO_3^- was added to the serosal fluid of the half-bladder from which data are depicted by solid circles. This produced no more change in I_{sc} than was produced by addition of a HCO_3^--free dummy solution to the control half-bladder from which data are depicted by open circles.

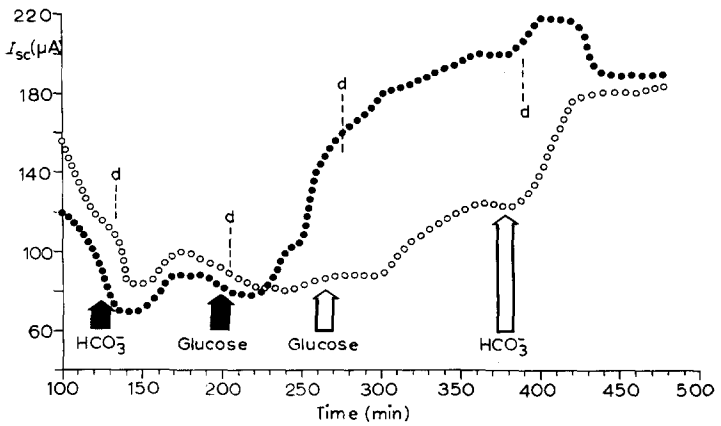

Fig. 3. Glucose dependence of the HCO_3^--induced acceleration of Na^+ transport.

At 200 min, glucose was added to the serosal fluid (final concn., 11 mM) of the HCO_3^--enriched bladder. After a time lag of 30 min, I_{sc} began to increase, going from 80 to 180 μA in the next 1 h and reaching maximal levels of 200–220 μA at 400 min. During the first hour following glucose enrichment, the period of rapid rise in I_{sc}, the I_{sc} of the control half-bladder (open circles) remained constant at a low level.

At 260 min, glucose was added to the serosal fluid (final concn., 11 mM) of the HCO_3^--free half-bladder. After a 30-min time lag, there occurred an increase in I_{sc} (from 80 to 120 μA) in the next hour. This glucose-induced increase was neither as rapid nor as great as that induced in the mated half-bladder primed with HCO_3^- (solid circles).

At 370 min, when the I_{sc} of the HCO_3^--free bladder system had reached a steady-state level, HCO_3^- was added to the serosal fluid (final concn., 17 mM). Within 10 min, the level of I_{sc} began to increase rapidly reaching a level of 180 μA at 420 min. It can be seen that both mated half-bladders ultimately delivered the same amount of I_{sc} regardless of the sequence of glucose and HCO_3^- enrichment. Similar effects were found in four other experiments of this type.

Evidently, HCO_3^- did not produce its effect in the absence of glucose after a 2–4 h-period of depletion of endogenous glycogen. These data suggest that the HCO_3^--induced acceleration of Na^+ transport is totally dependent on the presence of available substrate. On the other hand, the glucose-induced acceleration was not an all-or-none phenomenon, since it did occur in the absence of ambient HCO_3^-.

Magnitude of Na^+ transport rates in the turtle bladder as reported from different laboratories

Data on Na^+ transport from bladders bathed in HCO_3^--rich fluids[3] were compared with similar data from bladders in HCO_3^--free fluids[4,5]. Such a comparison, on a cm^2 basis, showed that the mean values for I_{Na+} and I_{sc} under HCO_3^--rich conditions were much greater in magnitude than those for I_{Na+} and I_{sc} under HCO_3^--free conditions. This discrepancy was explained only in part by data of this report showing the effect of serosal HCO_3^-. A large discrepancy between the magnitude of current densities measured by us and that measured by others[4,5] under apparently similar conditions still remains. For example, in HCO_3^--free NaCl Ringer solution buffered by phosphate, our mean values for I_{Na+} and I_{sc} were 70 and 66 $\mu A/cm^2$, respectively; and the mean values for I_{Na+} and I_{sc} found by others were 37 and 35 $\mu A/cm^2$, respectively[5]. These discrepancies may be attributed to the chamber design used in our experiments which exploited the high jetting velocity of circulating ambient fluids tangential to the bladder surfaces in order to maximize the rate of O_2 delivery and to minimize unstirred layer effects at the surfaces.

DISCUSSION

Whereas present data clearly demonstrate a dependence of Na^+ transport on the presence of serosal HCO_3^- (and/or Cl^-), they are perfectly consistent with previous data demonstrating an independence of Na^+ transport from the presence of mucosal HCO_3^- (and/or Cl^-). In essence, as long as the anionic composition of the serosal fluid is kept constant (*e.g.*, either HCO_3^--rich or HCO_3^--free), the rate of Na^+ transport will not be affected by variations in the mucosal anions.

Although serosal HCO_3^- at 17–20 mM produced a clear effect when compared with no serosal HCO_3^-, present experiments were not designed to yield the kinetic pattern of increase in Na^+ transport *versus* concentration of serosal HCO_3^- (or Cl^-). However, preliminary data suggest that a minimal concentration, 2–4 mM, is apparently required before any effect on Na^+ transport is detectable.

The fact that HCO_3^- acts after its addition to the serosal rather than to the mucosal fluid has a bearing on the site of the HCO_3^- receptor. One can infer from these data that HCO_3^- could interact with any site in the Na^+ transport pathway except that at the interface between the mucosal membrane and the mucosal fluid. However, when HCO_3^- itself is being transported from mucosa to serosa (rather than acting as a stimulus for Na^+ transport), it apparently moves along a transport pathway in such a way that it does not interact with the HCO_3^--sensitive site which accelerates Na^+ transport. The precise cellular location of the anion-sensitive, transport-accelerating site for Na^+ remains unknown. It could be located on either surface of the serosal membrane, on any of the cellular organelles or even at the interface between the mucosal membrane and the cytoplasm.

Wherever located, the HCO_3^- effect on Na^+ could be due to a nonspecific anionic interaction with the transport accelerator, to a nonspecific buffering effect of the HCO_3^- or to some specific action of HCO_3^- *per se*. The first possibility, a nonspecific anion effect, is consistent with data herein showing that serosal Cl^-, like HCO_3^-, accelerates Na^+ transport. This suggests that at least part of the effect of HCO_3^- could be due to its anionic nature and the remainder of the effect to its buffering property.

Recently, FUNDER *et al.*[15] have suggested that HCO_3^- protects against the inhibitory effect of high pCO_2 on Na^+ transport, presumably by preventing the decrease in cellular pH induced by increasing the ambient pCO_2. This effect, consistent with an intracellular buffering effect of HCO_3^-, might be shared by other buffer anions if the cell membrane were as permeable to them as it apparently is to HCO_3^-.

However, in preliminary experiments, the zwitterionic amino acid buffers, 2-(*N*-morpholino)ethane sulfonic acid ($pK_a = 6.15$) and *N*-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid ($pK_a = 7.5$), were added to the serosal fluid[16]. No sustained increase of Na^+ transport was elicited. Either the buffering property alone is not sufficient to account for the HCO_3^- effect or few if any non-HCO_3^- buffers can penetrate the cell membrane[17].

Whatever the nature of HCO_3^- interaction with the Na^+ transport system, the presence and utilization of glucose is apparently an absolute requirement for HCO_3^--induced acceleration of Na^+ transport. On the other hand, O_2 is not an absolute requirement for the accelerating effect.

Thus, the HCO_3^--induced acceleration of transport could be induced in anoxic as well was in oxygenated bladders.

The HCO_3^--dependence of the O_2-induced recovery of Na^+ transport from near-zero levels after 2 h of nitrogenation introduces a new and interesting aspect of the transport system in the turtle bladder. Although Na^+ transport proceeds in a HCO_3^--free bathing system fortified by glucose and O_2, it cannot be started from zero levels by O_2 even in the presence of glucose. Apparently serosal HCO_3^- is a required transport accelerator from zero levels as well as from finite levels of Na^+ transport in a glucose-enriched bladder system.

Problems remaining pertain to the specific portion or portions of the pump–carrier transport system affected by serosal HCO_3^-. Suffice it to say that HCO_3^- could engage with any one or all the portions of the pump–carrier system. In this connection, current work is directed toward a study of the anionic sensitivity of microsomal ATPase activity and toward the substrate-specificity of the HCO_3^- effect on transport.

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