ACID-BASE CHANGES ACROSS THE WALL OF HAMSTER AND RAT INTESTINE

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Acid-base relationships within the lumen of the small intestine are of considerable interest since they influence not only the rate of breakdown of foodstuffs but also the absorption of a variety of substances. Many pH measurements of intestinal contents of fasted and fed animals have been made[1–4], but the contamination by gastric and pancreatic juices as well as food makes the contribution of the small intestine to the observed values difficult to assess. The first extensive study of the pH and ionic content of secretions from isolated loops of intestine was performed by DE BEER, JOHNSTON AND WILSON[5]. In the unanesthetized dog the jejunal secretion had a pH of about 6.8 and a total CO_2 of about 20 mmolar; the ileal secretion had a pH of 7.6 and a total CO_2 of about 80 mmolar. They also noted that the calculated CO_2 tension was higher in the lumen of the jejunum than in the blood. Similar results were obtained in human jejunum by MCGEE AND HASTINGS[6].

When various solutions are instilled into the lumen of the small intestine and then removed after different time intervals characteristic pH and bicarbonate changes are observed. In the dog[7–10], rat[7] and man[11] solutions placed in the jejunum become slightly acid with respect to blood and similar solutions in the lower ileum become alkaline.

It has been shown previously[12] that during glucose absorption by rat intestine *in vitro* the mucosal side of the jejunum or upper ileum became acid while the pH of the solution of the serosal side remained practically unchanged. In one series of experiments, 85% of the acid produced could be accounted for as lactic acid. Preliminary experiments have also been reported[12] indicating bicarbonate secretion *in vitro* by the lower ileum of the hamster.

The functions of the small intestine are so complex that attempts have been made to simplify the experimental procedure in order to separate partially some of the transport processes. The experiments to be reported were carried out with sugar-free saline solutions containing only a few ions. Under these conditions, an *in vitro* preparation of the small intestine is capable of developing concentration gradients of a number of ions across its wall.

METHODS

Unfasted Sprague-Dawley rats and golden hamsters were used in this work. The animals were killed by a blow on the head and the intestine washed out carefully *in situ* with isotonic saline. The small intestine was everted with a long probe, segments (5–10 cm in length) filled with fluid using

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a blunt needle attached to a syringe, and tied at both ends, as previously described[13]. Care was taken not to over-distend the small sacs with fluid. Everted sacs were incubated in 50 ml Erlenmeyer flasks fitted with two-hole rubber stoppers into which were inserted short glass tubes for gassing the flask. The Krebs-Henseleit bicarbonate-saline solution[14] was used without the addition of either magnesium sulfate of calcium chloride and gassed with 5% CO_2 and 95% O_2.A small amount of phenol red was present in all solutions in order to visualize the pH changes. Most incubations were carried out at 30° C (a few at 37° C).

Total CO_2 was determined by the method of VAN SLYKE AND NEILL[15], and pH with a glass electrode (Beckman model G) at 25° C. The correction of the pH measured at 25° C for 30 and 37° C was found experimentally to be less than 0.05 pH units and was not applied to the measured values. The solution on the mucosal side of the intestine was poured from the flask into a pH cell and immediately covered with a layer of liquid petrolatum; the solution inside the sac of intestine (serosal side) was drained from the cut end of the sac into a cell and likewise layered with oil. Although there was probably a slight loss of CO_2 from the solutions during the 1–2 seconds exposure to air, this was not found to affect appreciably the CO_2 tension in control samples gassed with 5% CO_2. Bicarbonate was also determined in the Warburg apparatus by tipping 0.5 ml of 5 N H_2SO_4 (side arm) into 2.5 ml of a bicarbonate-containing solution (main compartment) previously gassed with 5% CO_2 and 95% O_2. The bicarbonate concentrations calculated from total CO_2 and pH measurements agreed within 5% with those measured in the Warburg apparatus in a series of ten experimental solutions.

Sodium and potassium were determined with the flame photometer (Perkin and Elmer) and chloride by the method of SCHALES AND SCHALES[16]. Phosphate[17] and lactate[18] were estimated colorimetrically.

RESULTS

Golden hamster

Preliminary experiments indicated that the presence of glucose in the incubating medium was not essential for the ion transport. It was omitted from all subsequent experiments since its presence was associated with two complicating effects: (1) movement of fluid from the mucosal to serosal side and (2) the formation of lactic acid.

When sacs of hamster ileum were incubated in bicarbonate-saline the concentration of bicarbonate fell on the serosal side and rose on the mucosal side. In Table I are shown the results obtained with two segments of ileum from each of two animals. The final volume on the mucosal side was about three times that on the serosal side so that the concentration changes were more marked on the latter side.

Associated with these changes in bicarbonate concentration there were also changes in pH. The pH on the mucosal side rose while that on the serosal side fell.

TABLE I

TOTAL CO_2 AND pH GRADIENTS DEVELOPED ACROSS THE WALL OF HAMSTER ILEUM AFTER INCUBATION IN BICARBONATE-SALINE

Sacs of hamster ileum were incubated in bicarbonate-saline (gassed with 5% CO_2 and 95% O_2) for 1 hour at 30° C. Initial volume on the mucosal side was 3.0 ml, on the serosal side 1 ml.

Experiment No.		*Sample*	*Total CO_2 (Van Slyke) mmoles/l*	*pH*
1	(a + b)	Initial	27.6	7.43
	(a)	Final mucosal	30.1	7.55
	(a)	Final serosal	15.6	7.13
	(b)	Final mucosal	33.2	7.58
	(b)	Final serosal	17.8	7.14
2	(a + b)	Initial	26.8	7.47
	(a)	Final mucosal	30.6	7.52
	(a)	Final serosal	14.6	7.25
	(b)	Final mucosal	31.3	7.49
	(b)	Final serosal	14.0	6.90

TABLE II

IONIC CHANGES ACROSS THE HAMSTER ILEUM *in vitro* (AEROBIC)

Sac taken from hamster ileum incubated in bicarbonate-saline (gassed with 5% CO_2 and 95% O_2) for 90 minutes at 30°C. Final wet weight of tissue 198 mg (dry weight 45 mg) *I* = initial; *F* = Final; *Δ* = Change

Solution	*Volume*	*Bicarbonate*		*Chloride*		*Phosphate*		*Lactate*		*Sodium*		*Potassium*	
		Concn.	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*
	ml	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*
I Mucosal	2.00	25.5	51.0	121	242	0.9	1.8	0	0	147	294	4.64	9.29
F Mucosal	2.08*	29.6	61.6	116	241	1.1	2.3	0.27	0.56	152	316	6.38	13.3
I Serosal	1.00	25.5	25.5	121	121	0.9	0.9	0	0	147	147	4.64	4.64
F Serosal	0.92	15.4	14.2	126	116	1.2	1.1	0.72	0.66	152	140	3.98	3.66
Δ Mucosal	+0.08*		+10.6		—1		+0.5		+0.56		+22		+4.0
Δ Serosal	—0.08		—11.3		—5		+0.2		+0.66		—7		—1.0

* Calculated from the three measured volumes, assuming no tissue swelling or evaporation.

TABLE III

IONIC CHANGES ACROSS THE HAMSTER ILEUM *in vitro* (ANAEROBIC)

Sac of hamster ileum (adjacent to segment in Table II) incubated in bicarbonate-saline (gassed with 5% CO_2 and 95% N_2) for 90 minutes at 30°C Final wet weight of tissue 158 mg (dry weight 25 mg) *I* = initial; *F* = final; *Δ* = change.

Solution	*Volume*	*Bicarbonate*		*Chloride*		*Phosphate*		*Lactate*		*Sodium*		*Potassium*	
		Concn.	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*	*Concn.*	*Total amount*
	ml	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*	*mmoles/l*	*μmoles*
I Mucosal	2.00	25.5	51.0	121	242	0.9	1.8	0	0	147	294	4.64	9.28
F Mucosal	2.05*	23.7	48.6	121	248	1.5	3.1	1.2	2.5	150	308	8.1	16.6
I Serosal	1.00	25.5	25.5	121	121	0.9	0.9	0	0	147	147	4.6	4.6
F Serosal	0.95	20.0	19.0	121	115	1.4	1.3	2.8	2.7	150	143	8.1	7.7
Δ Mucosal	+0.05*		—2.4		+6		+1.3		+2.5		+14		+7.3
Δ Serosal	—0.05		—6.5		—6		+0.4		+2.7		—4		+3.1

* Calculated from the three measured volumes, assuming no tissue swelling or evaporation.

The final concentration gradient of hydrogen ions across the wall of the intestine was between 2 and 3. The calculated CO_2 tensions in the solutions showed some variations from one experiment to another but on the whole the final serosal solution had a higher CO_2 tension than that on the mucosal side. This is discussed in more detail later.

There was a marked difference in the ability of the upper and lower segments of the small intestine to secrete bicarbonate. Fig. 1 shows that the upper jejunum transported only a small amount while the lower ileum transported a much larger amount, with the upper ileum intermediate. There was good agreement between the μmoles of bicarbonate lost from one side and the μmoles gained on the opposite side. This was particularly true in the case of ileal segments where the agreement was usually within 10%.

A more detailed study of the ion changes across sacs of ileum was made both aerobically and anaerobically (see Tables II and III). Aerobically there was a movement of both fluid and bicarbonate from serosal to mucosal side. Associated with the change in concentration of bicarbonate there was a change in the opposite direction of the chloride concentration, a rise on the serosal and fall on the mucosal side. There was, however, no appreciable net movement of chloride across the wall in either direction. A slight loss of phosphate from the tissue was observed. The very small amount of lactic acid production did not contribute a significant amount of acid to the solution on either side of the intestine. Sodium ions were secreted from the serosal to mucosal sides. Since there is an experimental error of about ± 2% in the determination of sodium concentration there would be an error of ± 6 μmoles in the value for the sodium change on the mucosal side. Likewise, there probably would be an error of about ± 3 μmoles in the sodium change on the serosal side. Owing to the rather large error of these values it is possible that the sodium lost from the serosal side may be approximately the same as that gained on the mucosal side. This would indicate the secretion of a solution of sodium bicarbonate from serosal to mucosal side.

In the aerobic experiment the potassium concentration fell on the serosal side and rose on the mucosal side. Although there was a loss of 3 μmoles of potassium from the tissue, there was a net transport of 1 μmole from serosal to mucosal side. This potassium transport though rather small in amount was consistently found with segments taken from the lowest ileum. Segments from higher regions of the small intestine did not show this effect.

Anaerobically (Table III) bicarbonate was lost from both sides and half of this acidity would be accounted for by the lactic acid production by the tissue. There was considerably greater loss of potassium anaerobically than aerobically. No concentration gradient was observed for this ion. The concentration changes which were observed with the other ions were not marked.

Segments of hamster intestine taken from the lowest ileum usually showed a movement of fluid from the serosal to mucosal sides (Table IV). Nine out of ten experiments showed this effect. The loss of fluid from the serosal side was not due to tissue swelling since determinations of initial and final wet and dry weights indicated only negligible increase in water content of aerobic tissue. It should be noted that segments of the intestine from the upper ileum and jejunum do not show this effect, but absorb fluid from the mucosal to serosal sides[19]. The bicarbonate concentration in the secreted fluid was calculated for each experiment and was found to vary considerably. The average bicarbonate concentration in eight experiments was 157 mmolar.

TABLE IV

BICARBONATE AND FLUID SECRETION BY LOWER ILEUM OF THE HAMSTER

Sacs taken from the lowest region of the hamster ileum were incubated in bicarbonate-saline (gassed with 5% CO_2 and 95% O_2) for 1 hour at 30°C. Fluid movement was measured as loss or gain in volume on the serosal side.

Experiment No.	*Movement from serosal to mucosal side*		*Concentration of HCO_3 in fluid mmoles/l*
	Fluid ml	*HCO_3 µmoles*	
1	0.08	19	238
2	0.13	11	85
3	0.10	15	150
4	0.03	12	400
5	0.08	6	75
6	0.08	11	138
7	0.06	12	200
8	0.05	13	260
9	—0.03	10	—
10	0.13	14	108
Average*			157

* Nos. 4 and 9 were not included in this average since volumes less than 0.05 ml were not considered significant.

TABLE V

CO_2 TENSION ON THE TWO SIDES OF HAMSTER INTESTINE *in vitro*

Sacs of hamster ileum were incubated in bicarbonate-saline (gassed with 5% CO_2 and 95% O_2) for 1 hour at 30°C or 37°C. CO_2 concentration calculated from pH and total CO_2 determinations by Henderson-Hasselbalch equation.

Temperature	*No. of experiments*	*Solution*	*CO_2 concentration mmoles/l*
30°C	6	Initial	1.14
	11	Serosal side	1.38*
	11	Mucosal	1.17*
37°C	5	Initial	1.16
	10	Serosal side	1.41**
	10	Mucosal side	1.12**

* Student's *t* test was applied to the paired experiments and *p* was less than 0.05.
** Student's *t* test was applied to the paired experiments and *p* was equal to 0.001.

No significance is attached to this absolute value; it is only given to indicate the relatively high bicarbonate concentration in the secreted fluid.

Since information concerning the CO_2 tension was considered of interest in relation to a variety of mechanisms of ion transport, a series of experiments were carried out to determine the CO_2 tension on each side of the gut wall. Total CO_2 and pH measurements were made and the CO_2 concentration calculated from the Henderson-Hasselbalch equation. The apparent pK_1 at 37° C was taken as 6.1; it was not corrected for temperature in the 30° C experiments since relative rather than absolute values were desired. Table V shows the mean CO_2 concentrations on the two sides following one hour incubation, as well as that for the initial solution. At 30° C the average CO_2 concentration on the serosal side was 18% higher than that on the mucosal side. The possibility that this result was due to chance alone was less than one in twenty. The observed CO_2 concentration inside the sac is the result of two processes, the production of CO_2 and its diffusion across the wall to the mucosal side. If CO_2 production within

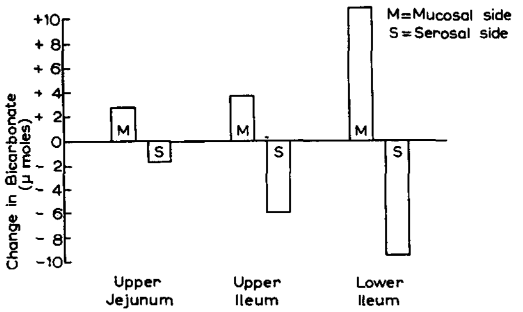

Fig. 1.

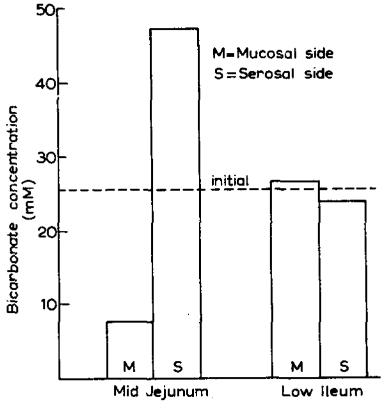

Fig. 2.

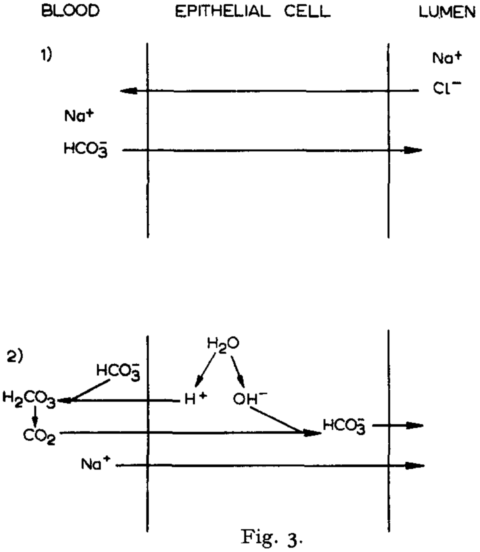

Fig. 3.

Fig. 1. Bicarbonate secretion at different locations along the small intestine of the hamster. Sacs of everted intestine (39, 44, and 43 mg dry weight respectively) incubated in bicarbonate-saline for 90 minutes at 30°C. Gas phase, 5% CO_2 and 95% O_2. Initial volume on mucosal side 2.0 ml, volume on serosal side 1.0 ml.

Fig. 2. Concentration gradients of bicarbonate developed across the jejunum and ileum of the rat. Sacs of everted intestine (50 and 43 mg dry weight respectively) were incubated in bicarbonate saline for 2 hours at 30°C. Gas phase, 5% CO_2 and 95% O_2. Initial volume on mucosal side 2.0 ml, volume on serosal side 0.9 ml.

Fig. 3. Two hypothetical mechanisms for bicarbonate secretion by hamster ileum.

the sac were related directly or indirectly to some metabolic process, then raising the temperature to 37° C should approximately double the rate of CO_2 production without affecting the rate of diffusion out of the sac by more than about 10%. In an attempt to increase the CO_2 concentration within the sac in this manner ten experiments were performed at 37° C. In all these experiments the CO_2 concentration was higher on the serosal than mucosal side, the average on the former side being 26% higher than on the latter side. It should be emphasized that the rate of loss of CO_2 by diffusion into the air during the handling of the solutions would be greater from the serosal solution which contains a higher CO_2 tension. The differences between the CO_2 tensions in the two solutions might in reality be greater than observed. The possible significance of these observations will be discussed later.

Rat

The upper small intestine of the rat showed an acid-base change opposite to that observed with hamster intestine. Fig. 2 shows the changes in bicarbonate concentration across the wall of two sacs of rat intestine. With the jejunal sac, the concentration fell from an initial value of 25.5 mmolar to 7.5 mmolar on the mucosal side and rose from 25.5 mmolar to 48.3 mmolar on the serosal side. The sac obtained from the low ileum showed a slight effect in the opposite direction. This effect, although small, was consistently found with segments from the lowest regions of rat ileum. It was assumed that the bicarbonate concentrations reflect changes in pH across the wall of the intestine.

It was found that with jejunal sacs more bicarbonate was lost from the mucosal side than appeared on the serosal side. In the jejunal experiment shown in Fig. 2 37 μmoles were lost from the mucosal side and only 28 appeared on the serosal side. This acid production was not due to lactic acid (which was measured chemically) nor to any detectable non-volatile acid which could be found with paper chromatography. A considerable amount of inorganic phosphate, however, was lost from the tissue during these experiments, which suggests the breakdown of organic phosphates within the cells. The hydrolysis of certain phosphates could contribute to the loss of bicarbonate observed.

These general results were confirmed in experiments carried out on anesthetized rats. Table VI shows that the bicarbonate-saline introduced into the jejunum for a period of from 10 to 30 minutes became acid and the total CO_2 fell to $^1/_4$ to $^1/_6$ the original value. A marked secretion of bicarbonate into the lumen was noted in the low ileum. The total CO_2 concentration doubled and the pH rose more than 0.3 of a unit. These *in vivo* experiments demonstrate the striking difference in acid-base changes across the wall of the upper and lower segments of the rat small intestine.

TABLE VI

pH CHANGES WHICH OCCUR WHEN BICARBONATE-SALINE IS PLACED IN RAT INTESTINE *in vivo*

Rats were anesthetized with nembutal, the abdomen opened and two loops of intestine washed out with saline. 2.00 ml of bicarbonate-saline were introduced into a loop of upper jejunum and a loop of lower ileum (each about 15 cm in length). The abdomen was closed for a period of time followed by re-exposure of the loops and aspiration of the contents.

Animal No.	*Location*	*Time minutes*	*Total CO_2 mmoles/l*	*pH*
Initial saline	—	—	27.5	7.45
1	Jejunum	30	4.8	6.55
	Ileum	30	63.4	7.80
2	Jejunum	20	4.0	6.47
	Ileum	20	60.1	7.82
3	Jejunum	20	4.6	6.62
	Ileum	20	67.7	7.89
4	Jejunum	10	7.3	6.65
	Ileum	10	56.8	7.78

DISCUSSION

The upper small intestine of most species of animals produces an acid secretion while the lower segment produces an alkaline one. The golden hamster appears to be unique among species tested since it produces an alkaline secretion throughout the entire length of its small intestine, although it is most active in the lower ileum. These lowest segments of the hamster ileum were found to be ideal for the study of bicarbonate secretion.

Possible mechanisms for bicarbonate secretion by hamster ileum

Alkaline secretion by this tissue may be similar in mechanism to that of HCl secretion by the stomach. In fact, a very striking similarity is found between the present experimental results and those of DAVIES[20] who used tied sacs of frog gastric mucosa incubated in bicarbonate-saline. Under these conditions, he found a loss of bicarbonate from the mucosal side and an equivalent gain on the serosal side. Among the mechanisms proposed for HCl secretion by the stomach is that of HOGBEN[21] (see Fig. 3, No. 1). This mechanism could account for the changes in bicarbonate observed with hamster ileum, but not the changes in sodium. Furthermore, there were no net changes in chloride. The most important argument against this hypothesis in the case of hamster ileum is that this mechanism does not involve hydrogen ions directly, and would not account for the high CO_2 tension found on the serosal side of the intestine.

A second type of mechanism (Fig. 3, No. 2) is that which involves the exchange of a hydrogen ion for a sodium ion across one cell membrane. In the *in vitro* experiments, the hydrogen ion would pass into the bicarbonate solution on the serosal side displacing an equivalent amount of CO_2. The CO_2 tension on this side would rise and the dissolved CO_2 would tend to diffuse across the wall. Experimental support for this portion of the mechanism is given in Table V. According to this scheme, the hydroxyl ion, formed by the splitting of water, would be neutralized by CO_2 either from that on the serosal side or by metabolic CO_2. The bicarbonate ion thus formed, along with a sodium ion, would be secreted onto the mucosal side of the intestine.

Secretions of the rat small intestine

The changes observed with the rat jejunum were opposite to those found in the hamster; the bicarbonate concentration fell on the mucosal side and rose on the serosal side. Although the rat intestine produced some acid material (perhaps owing to breakdown of organic phosphates) there was a net movement of bicarbonate from mucosal to serosal sides. This latter effect is similar to that observed during HCl secretion by frog gastric mucosa[20]. The movement of other ions across the rat jejunum under these conditions has not been studied in detail so that it is not possible at present to state whether there is a secretion of chloride as there is with gastric mucosa.

The ileum of the rat secreted a bicarbonate solution into the lumen. This was clearly shown *in vivo* although *in vitro* preparations were considerably less active in this respect.

Acid secretion by dog and human jejunum

The secretions from the jejunum of the dog are similar to the present observations on the rat jejunum; in each case the mucosal solution becomes slightly acid. One feature of the jejunal secretion of the dog deserves special comment. DE BEER, JOHNSTON AND WILSON[5] state: "If one calculates the CO_2 tension from our data, one obtains, in some instances, values far higher than those obtained for blood." This was confirmed by HERRIN[22]. An extensive study of CO_2 tension in the jejunal secretions of man was made by MCGEE AND HASTINGS[6] who found values between 60 and 200 mm of Hg. They suggested two types of secretions, one containing bicarbonate and one containing acid. If one dismisses the possibility of the secretion of CO_2 gas by the epithelium, one must conclude that the high CO_2 tension was due to displacement of bicarbonate by hydrogen ions.

It is therefore possible to explain both the bicarbonate secretion by hamster ileum and acid secretion by the jejunum of dog and man by similar mechanisms—one which involves the exchange of hydrogen ion with some other cation across one border of the epithelial cell. This type of mechanism has been shown to occur in the distal tubule cells of the kidney during acid secretion [23] and in yeast during potassium uptake from the surrounding medium[24]. The ultimate origin of the hydrogen ion generated by the intestine is probably water, as suggested by DAVIES[20] for HCl secretion by the stomach.

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SUMMARY

1. When small sacs of hamster small intestine were incubated in bicarbonate-saline gassed with 5 %CO_2 and 95 % O_2, the bicarbonate concentration and pH rose on the mucosal side while the reverse affect was noted on the serosal side. This concentration gradient of bicarbonate ions was completely inhibited anaerobically.

2. In the lowest segment of the ileum a solution containing high concentrations of sodium bicarbonate was secreted from serosal to mucosal side. There was no appreciable movement of chloride ion across the wall under these conditions.

3. The concentration of CO_2 was found significantly higher on the serosal than mucosal side. This evidence has been used to support the hypothesis involving an exchange of hydrogen ion for sodium ion across the serosal surface of the mucosal cell.

4. Sacs of rat jejunum showed a fall in bicarbonate concentration on the mucosal side and a rise on the serosal side; sacs taken from the lower region secreted a small amount of bicarbonate from serosal to mucosal sides.

5 When bicarbonate-saline was placed in a washed loop of jejunum of an anesthetized rat the total CO_2 and pH fell; a similar solution in a loop of ileum of the same animal showed a rise in total CO_2 and pH.

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