Concentration Gradients of Lactate, Hydrogen and some other Ions Across the Intestine *in vitro*

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Previous work (Wilson & Wiseman, 1953b) has shown that 50–80 % of the glucose which disappears from the mucosal side of sacs of rat intestine *in vitro* is converted into lactic acid and that the lactate appears in a higher concentration on the serosal than on the mucosal side. The experiments to be presented in this paper show that the hydrogen and lactate ions produced by the mucosa migrate in different directions, the hydrogen ions appearing on the mucosal side and the lactate ions mainly on the serosal side. A study has also been made of the associated movements of bicarbonate, chloride, sodium and potassium ions. A preliminary communication of this work has appeared elsewhere (Wilson, 1953).

EXPERIMENTAL

In vitro preparation of rat intestine (according to the method of Fisher & Parsons). Oxygenated bicarbonate-saline (Krebs & Henseleit, 1932) containing 0.5 % (w/v) glucose was circulated on each side of a segment (60 cm.) of rat small intestine, which had previously been washed with glucose-free bicarbonate-saline, according to the method of Fisher & Parsons (1949a). The inner circuit (mucosal side) contained 65 ml. of bicarbonate-saline with a hydrostatic pressure of 30 cm. of water; the outer circuit (serosal side) contained 50 ml. Some glucose-free washing saline remained in the lumen of the intestine at the time it was attached to the circulation unit with a consequent dilution of the glucose in the 65 ml. of added bicarbonate-saline. An initial sample (15 ml.) was removed from both inner and outer solutions after circulation for 2 min., the time required for complete mixing. The initial volume on each side was calculated from the dilution of the glucose in the saline and the volume of fluid added to each side. After 1 hr., 15 ml. samples were removed from both circuits for analysis, the remaining solution was immediately drained and the circuits were washed out with glucose-free saline. The final volume was calculated from the glucose concentration in the 1 hr. sample and the total amount of glucose in the remaining saline plus washings.

The length of the relaxed intestine was measured at the end of each experiment before mincing. To enable calculations on the basis of dry wt., sixteen experiments were performed in which the dry wt. and length were measured. The average dry wt. was 10.0 mg./cm. intestine (range 7.0–13; s.d. 1.7). No difference was found between jejunal and ileal segments.

Sacs of everted intestine. Samples of rat or golden hamster intestine from 5 to 10 cm. in length were everted, filled with fluid (usually 1.0 ml.) with a blunt needle attached to a syringe and tied at both ends as previously described (Wilson & Wiseman, 1953a, b). The tissue was kept in ice-cold saline during the preparation of the sacs. Large conical Warburg flasks (125 ml.) were used for the incubation of the sacs in most aerobic and all anaerobic experiments. In a few aerobic experiments, 150 ml. flasks of the type described by Krebs (1933) which were gassed continuously replaced the Warburg flasks. Bicarbonate-saline (Krebs & Henseleit, 1932) containing 0.3 % (w/v) glucose was used for the suspending medium (mucosal side) and for the fluid inside the sac (serosal side). The flasks were gassed with 5 % CO₂ + 95 % O₂ aerobically and 5 % CO₂ + 95 % N₂ anaerobically. Phosphorus was placed in the centre well of the Warburg flask in anaerobic experiments.

The initial volume on the serosal side was that injected into the sac after careful blotting of the open end. The volume of fluid in the sac after incubation was determined by weighing the sac before and after emptying. The final volume on the mucosal side was calculated from the difference between the initial volume on the mucosal side and the gain in fluid on the serosal side.

Chemical methods. Glucose was measured by the method of Hulme & Narain (1931) on Cd(OH)₂ filtrates (Fujita & Iwatake, 1931) in experiments with the Fisher & Parsons apparatus and by the method of Nelson (1944) in all others. Lactic acid was estimated by the method of Barker & Summerson (1941), inorganic phosphate by the method of Berenblum & Chain (1938). Chloride was determined by the Volhard procedure. Bicarbonate was determined manometrically by equilibrating 0.5 ml. bicarbonate solution and 2.0 ml. water with 5 % CO₂ + 95 % O₂ and adding 0.5 ml. 3N-H₂SO₄ from the side arm. Sodium and potassium were measured with the flame photometer of Domingo & Klyne (1949). The pH was calculated from the measured bicarbonate concentrations, assuming 5 % CO₂ (1.14 mm) as gas phase and apparent pK₁ of carbonic acid as 6.10. It was assumed that this concentration of CO₂ was in equilibrium with the bicarbonate solution on each side of the intestinal wall.

RESULTS

Rat intestine (method of Fisher & Parsons)

Lactate and bicarbonate gradients. As shown in Table 1, about one-half of the glucose which disappeared from the mucosal side in five experiments appeared on the serosal side and about one-quarter was converted into lactic acid. The total amount of lactate found on the serosal side was 4–10 times that on the mucosal side. Table 2 shows the con-

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Table 1. *Glucose transport and lactate production in rat intestine (method of Fisher & Parsons)*

(Bicarbonate-saline containing about 0.5% glucose was circulated on the two sides of the intestine for 1 hr. at 38°. Gas phase 5% CO₂ + 95% O₂.)

Expt. no.	Glucose lost from mucosal side (μmoles)	Glucose transported to serosal side (μmoles)	Glucose converted into lactate (μmoles)	Lactate on serosal side (μmoles)	Lactate on mucosal side (μmoles)
1	580	270	176	273	79
2	811	547	180	322	38
3	1065	440	256	468	44
4	1088	475	259	450	68
5	600	339	234	391	77

Table 2. *Concentrations of bicarbonate and lactate developed across rat intestine (method of Fisher & Parsons)*

(Bicarbonate-saline containing about 0.5% glucose was circulated on the two sides of the intestine for 1 hr. at 38°. Gas phase 5% CO₂ + 95% O₂. pH calculated taking pK of carbonic acid = 6.10 and CO₂ = 1.14 mm. Two other experiments gave similar results.)

Expt. no. ...	Lactate concn. (mm)			Bicarbonate concn. (mm)			pH (calculated)		
	1	2	3	1	2	3	1	2	3
Mucosal side (initial)	0	0	0	23.7	22.5	27.3	7.42	7.39	7.48
Mucosal side (final)	1.53	1.07	1.10	16.6	13.2	12.0	7.26	7.16	7.02
Serosal side (initial)	0	0	0	24.0	24.3	30.4	7.42	7.43	7.53
Serosal side (final)	6.60	6.33	10.2	23.0	25.0	30.0	7.46	7.44	7.52

Table 3. *Comparison between lactate produced and bicarbonate decomposed in rat intestine (method of Fisher & Parsons)*

(Bicarbonate-saline containing about 0.5% glucose circulated on the two sides of the intestine for 1 hr. Lactate and bicarbonate estimated in the solutions on each side of the intestine, lactate only estimated in the gut wall.)

Gut no.	Length of intestine (cm.)	Lactate produced			Bicarbonate lost Mucosal and serosal sides (μmoles)	Lactate produced/bicarbonate lost (%)
		Mucosal and serosal sides (μmoles)	Gut wall (μmoles)	Total (μmoles)		
1	68	352	77	429	479	90
2	62	360	45	405	356	113
3	85	512	99	611	966	63
4	67	518	98	616	922	67
5	61	468	105	573	550	104
Mean	—	—	—	—	—	87

Table 4. *Movement of substances across the perfused rat intestine (method of Fisher & Parsons)*

(Bicarbonate-saline containing glucose circulated on the two sides of rat intestine (62 cm. in length; 620 mg. dry wt.) for 1 hr. at 38°. See Tables 1 and 2 (Expt. 1) for bicarbonate and lactate values in this experiment.)

	Volume (ml.)	Glucose		Sodium		Potassium		Phosphate		Chloride	
		Concn. (mm)	Total amount (μmoles)	Concn. (mm)	Total amount (μmoles)	Concn. (mm)	Total amount (μmoles)	Concn. (mm)	Total amount (μmoles)	Concn. (mm)	Total amount (μmoles)
Mucosal side (initial)	60.5	24.2	1460	148	8940	6.14	371	1.18	71.5	130	7860
Mucosal side (final)	51.7	17.0	880	146	7550	6.75	349	1.39	72	136	7040
Serosal side (initial)	35.8	27.7	990	141	5040	6.09	218	1.19	42.6	128	4580
Serosal side (final)	41.4	30.4	1260	140	5810	5.16	214	1.40	58.1	118	4890
Change on mucosal side	-8.8	—	-580	—	-1390	—	-22	—	+1	—	-820
Change on serosal side	+5.6	—	+270	—	+770	—	-4	—	+15	—	+310

centration gradients of lactate and bicarbonate developed across the intestinal wall. The bicarbonate concentration on the serosal side did not change appreciably, in spite of the presence of the large amount of lactate; the concentration on the mucosal side fell to about half its initial value, although only a small amount of lactate was present on this side. A striking feature of these experiments is that while most of the lactate ions appeared on the serosal side virtually all of the acidification occurred on the opposite side.

A comparison was made between the lactate produced and the bicarbonate decomposed to determine whether any other acid was formed.

To estimate the lactate in the gut wall, the entire intestine after 1 hr. in the circulation unit was finely minced in a tissue blender with an ice-cold solution of CdSO₄. Lactate was estimated in the protein-free filtrate after copper/lime treatment. Filtrates prepared from intestines removed from two rats and immediately minced in the cold served as controls.

The average concentration found in the intestine after 1 hr. in the circulation units was 30 mm. The intestine removed directly from the animal contained 6.9 mm. No information is available on the bicarbonate content of the gut wall owing to the experimental difficulty of estimating bicarbonate in the tissue during incubation. Table 3 shows that an average of 87% (from 63 to 113%) of the acid production (bicarbonate displaced) may be accounted for as lactate.

Shapiro (1952), on the basis of experiments with a somewhat different *in vitro* method, showed that large amounts of some substance other than lactate is formed by the rat intestine but this was not found in the present work. Of the glucose which disappeared (Table 1, Expt. 1) from the mucosal side (580 μ moles), about half was transported across the wall (270 μ moles) and one-quarter converted into lactic acid (176 μ moles). Fisher & Parsons (1953) have shown that 0.165 mg. glucose/cm. of intestine accumulates in the wall under conditions used in these experiments. The 68 cm. of intestine used in this experiment would have accumulated 62 μ moles of glucose, assuming the value of Fisher & Parsons given above. Assuming a Q_{O_2} of -20 (Wilson & Wiseman, 1953c) and a R.Q. of 1.0 (and 10 mg. dry wt./cm. of intestine), 101 μ moles of glucose would be completely oxidized. The total glucose accounted for in this way (609 μ moles) agrees fairly well with the measured glucose disappearance (580 μ moles). Similar results were obtained from other experiments.

Movement of other substances across the intestine. Results on the movement of water, glucose, sodium, potassium, phosphate and chloride are shown in Table 4. It might be thought that the movement of fluid across the intestinal wall, in this experiment,

Table 5. *Movement of substances across the wall of a sac of everted rat intestine*

(Sac of everted ileum incubated for 105 min. with bicarbonate-saline in 150 ml. flat-bottomed flask, gassed continuously with 5% CO ₂ + 95% O ₂ saturated with water. 276 mg. wet wt., 50.7 mg. dry wt.)												
Volume (ml.)	Glucose		Bicarbonate		Lactate		Chloride		Sodium		Potassium	
	Concn. (mM)	Total amount (μmoles)	Concn. (mM)	Total amount (μmoles)	Concn. (mM)	Total amount (μmoles)	Concn. (mM)	Total amount (μmoles)	Concn. (mM)	Total amount (μmoles)	Concn. (mM)	Total amount (μmoles)
Mucosal side (initial)	11.7	298	24.3	620	0	0	123	3140	154	3930	5.72	146
Mucosal side (final)	4.92	117	14.7	350	6.40	149	127	3020	154	3660	6.66	158
Serosal side (initial)	11.7	11.7	24.3	24.3	0	0	123	123	154	154	5.72	5.7
Serosal side (final)	16.9	45.1	24.4	65.0	56.0	150	80	214	147	392	5.58	14.9
Change on mucosal side	—	- 181	—	- 270	—	+ 149	—	- 120	—	- 270	—	+ 12
Change on serosal side	—	+ 33.4	—	+ 40.7	—	+ 150	—	+ 91	—	+ 238	—	+ 9.2

* Calculated values. See text.

as shown by the increase in volume on the serosal side (5.6 ml.), was due to the pressure of 30 cm. of water on the mucosal side, but since similar movements of water occur in sacs of everted intestine (see Table 5) where the pressure relationships are reversed it may be taken that the hydrostatic pressure in this preparation is not a major cause of the water movement. The loss of water of 3.2 ml. is partly due to swelling of the tissue (Fisher & Parsons, 1953) and partly due to systematic errors in the calculations of the volumes.

The concentration gradient of glucose developed across the wall and the quantity of glucose transported are similar to those reported by Fisher & Parsons (1949*a, b*).

There was a net movement of sodium across the wall of the intestine without any appreciable change in concentration on the two sides. There was no transport of potassium; the concentration on the mucosal side increased owing to the loss of water and decreased on the serosal side owing to the gain in fluid. There was a small net loss of potassium from both solutions, presumably due to an uptake of this ion into the tissue. In the two other experiments in which potassium was estimated, a similar small loss from the solutions was noted. There was a gain in phosphate on both sides indicating loss from the tissue. The decrease in chloride concentration on the serosal side and the corresponding increase on the mucosal side is presumably partly connected with the unequal distribution of lactate.

Sacs of rat intestine

Lactate and bicarbonate gradients. The development of concentration gradients in sacs of everted intestine has already been described (Wilson & Wiseman, 1953*b*). In the experiment recorded in Table 5 the final concentration gradient of lactate was 9. Since the volume of fluid on the mucosal side was 9 times that on the serosal side, the total amounts of lactate on the two sides were equal. The final concentration of lactate on the serosal side was 56 mM, comprising more than one-third of the total anions. In spite of this large amount of lactate, the concentration of bicarbonate remained unchanged.

If lactic acid instead of lactate ions had been liberated on the serosal side, all of the bicarbonate would have been decomposed and the pH would have fallen to below 6.0. Instead, the pH remained about 7.4. The bicarbonate concentration on the mucosal side fell to about half its initial value. The total lactate produced on the two sides was 299 μ moles, while the total amount of bicarbonate displaced was 229 μ moles. The discrepancy between these values may be due to buffering of the acid by the tissue.

The $Q_{\text{glucose transported}}$ in the same experiment was 3.0 (μ l./mg. dry wt./hr.; 22.4 μ l. = 1 μ mole) and the $Q_{\text{glucose metabolized}}$ was 13. In the five experiments with the method of Fisher & Parsons (Table 1) the average $Q_{\text{glucose transported}}$ was 9 and the average $Q_{\text{glucose metabolized}}$ was 10. The $Q_{\text{glucose transported}}$ values for sacs of rat intestine vary from 2 to 7 (see Wilson & Wiseman, 1953*b*) but were always lower than those obtained with the circulation units of Fisher & Parsons.

Sodium and chloride ions moved across the intestine in a similar manner to that found with the Fisher & Parsons technique. No significant difference in concentration of sodium or potassium was noted on the two sides. There was, again, a large fall in chloride concentration on the serosal side and a rise on the mucosal side, which may be secondary to the unequal distribution of lactate. Potassium was lost from the tissue into both inner and outer solutions in contrast with the results presented with the other preparation of intestine. Three similar experiments (one sac from the jejunum and two from the upper ileum) gave essentially similar results. In two of these, inorganic phosphate was also estimated. There was a loss of phosphate from the tissue of 0.064 and 0.035 μ mole/mg. dry wt./hr. No difference in phosphate concentration was found on the two sides. This agrees with the findings of Smyth & Whaler (1953).

Two experiments were performed to test the effect of glucose on the movement of fluid (Table 6). In the absence of glucose there was practically no movement of fluid from mucosal to serosal sides. This is similar to the results obtained under anaerobic conditions in the presence of glucose (Wilson & Wiseman, 1953*b*).

Table 6. *The effect of glucose on the movement of water across sacs of everted intestine*

(The sacs were placed in 25 ml. Warburg flasks in Expt. 1 and in 125 ml. flasks in Expt. 2. Incubation for 90 min. at 37°.)

Expt. no.	Animal	Initial glucose concn. (mg./100 ml.)	Tissue	Dry wt. (mg.)	$Q_{\text{lactic acid}}$ (μ l./mg. dry wt./hr.)	Serosal side		Water transported (μ l. fluid/mg. dry wt./hr.)
						Initial volume (ml.)	Final volume (ml.)	
1	Hamster	300	Jejunum	29	—	0.370	0.875	11.6
		None	Ileum	32	—	0.520	0.576	1.2
2	Rat	210	Ileum	140	27	1.00	2.67	8.0
		None	Jejunum	122	3	1.00	1.03	0.2

Table 7. *The effect of anoxia on concentration gradients of lactate and hydrogen ions across sacs of everted rat intestine*

(Sacs of rat ileum were incubated in bicarbonate-saline containing 0.2% glucose for 90 min. at 37°. The initial bicarbonate concentration was the same on both sides of intestine.)

Gas phase	Initial bicarbonate concentration (mm)	Serosal side				Mucosal side				Total lactate produced (μmoles)	Total bicarbonate lost (μmoles)
		Final concn. (mm)		Bicarbonate + lactate	Final concn. (mm)		Bicarbonate + lactate				
		Bicarbonate	Lactate		Bicarbonate	Lactate					
5% CO ₂ + 95% O ₂	25.2	32.0	18.0	50.0	18.7	0.9	19.6	68	111		
5% CO ₂ + 95% O ₂	20.8	28.0	45.2	73.2	8.6	7.1	15.7	252	204		
5% CO ₂ + 95% N ₂	25.2	15.8	7.9	23.7	21.6	1.8	23.4	48	89		
5% CO ₂ + 95% N ₂	24.3	1.9	22.2	24.1	17.8	1.5	19.3	77	175		

Anaerobic experiments. Anaerobically the concentration of bicarbonate fell on the serosal side and this fall was roughly equivalent to the rise in lactate concentration, the sum of the two concentrations being equivalent to the initial bicarbonate concentration (Table 7). A lactate gradient appeared anaerobically, similar to that found aerobically.

Sacs of golden hamster intestine

The hamster produces considerably less lactic acid under aerobic conditions than does the rat (Wilson & Wiseman, 1953c), but the concentration gradients of lactate across the wall were similar to those of rat intestine (Table 8). In both sacs the concentrations of bicarbonate fell to about half the initial values on the serosal sides while they increased on the mucosal sides. In this respect the hamster differs from the rat intestine. There is a point of special interest in the case of the ileal sac. Bicarbonate (29 μmoles) was transported from the serosal side to the mucosal side against a concentration gradient of more than 2. Three further experiments showed a similar transport of bicarbonate.

Table 9 shows a typical experiment in which the transport of added lactate from mucosal to serosal sides was tested. There is no evidence for the transport of lactate against a concentration gradient; 4 μmoles of lactate were formed by the tissue and appear in both solutions. In the same sac, glucose was transported against a concentration gradient.

DISCUSSION

The lactate and hydrogen-ion gradient across the intestine. The experiments with rat intestine show that glucose is metabolized in the intestinal wall to lactic acid, the lactate ion appearing mainly on the serosal side and the hydrogen ion appearing on the mucosal side. The gradient for hydrogen ions is completely abolished by anaerobic conditions, though the lactate gradient persists under the conditions of the sac experiments. Concentration gradients of lactate have been previously demonstrated across the wall of tied sacs of frog gastric mucosa (Davies, 1948; Davenport, 1952).

A concentration gradient of a weak acid may be explicable by simple physical forces if the membrane is much more permeable to the undissociated acid than to the ionized form and if there is a pH difference across the membrane (see Höber, 1945). This hypothesis, however, cannot explain the lactate gradient across the rat intestine since the pH difference (0.3 unit) is too small to give rise to the observed lactate gradients of 4 to 20. Furthermore, the pH difference observed with sacs of hamster intestine are opposite to those which would favour a gradient of lactate by this kind of mechanism.

Table 8. *Lactate and bicarbonate gradients developed across sacs of everted hamster intestine*

(Intestine incubated with bicarbonate-saline containing 0.2% glucose in 125 ml. Warburg flasks for 100 min. at 37°. Initial volume on the serosal side = 2.0 ml.; initial volume on the mucosal side = 10.0 ml.)

	Jejunum		Ileum	
	Serosal side	Mucosal side	Serosal side	Mucosal side
Initial bicarbonate concn. (mM)	23.9	23.9	23.9	23.9
Final bicarbonate concn. (mM)	11.6	25.0	10.8	25.9
Final lactate concn. (mM)	5.32	0.91	1.38	0.36
Change in bicarbonate (μ moles)	-21	+1	-29	+29
Change in lactate (μ moles)	+12.7	+8.8	+2.1	+3.7
Total lactate formed (μ moles)		21.5		5.8
Total bicarbonate lost (μ moles)		20.0		0.0

Table 9. *Lactate gradient developed across a sac of everted hamster intestine after addition of lactate*

(Hamster ileum (22 mg. dry wt.) incubated in bicarbonate-saline in Warburg flask for 90 min. 37°. Gassed with 5% CO₂ + 95% O₂. The initial concentrations of DL-lithium lactate (6.9 mM) and glucose (5.1 mM) were the same on both sides of the intestine.)

	Serosal side	Mucosal side
Lactate		
Final concn. (mM)	10.1	7.9
Initial (μ moles)	3.1	20.8
Final (μ moles)	5.3	22.9
Change (μ moles)	+2.2	+2.1
Glucose		
Final concn. (mM)	14.8	1.83
Initial (μ moles)	2.3	15.1
Final (μ moles)	7.7	5.4
Change (μ moles)	+5.4	-9.7

A possible explanation for the gradient of lactate is the assumption that it is produced by the muscular layer and diffuses more rapidly into the solution on the serosal side of the small intestine. The following data show that this hypothesis is not tenable. The aerobic glycolysis of the smooth muscle of the cat small intestine is of the order of 2 μ l./mg. dry wt./hr. (T. H. Wilson, unpublished observations), which is similar to the values obtained in smooth muscle of other organs (Rosenthal & Lasnitzki, 1928). This is only 10–20% of the lactic acid production of the intestinal wall in the present experiments, the major site of the aerobic lactic acid production being the mucosa (Dickens & Weil-Malherbe, 1941).

A question arises whether the lactic acid produced by the mucosa diffuses primarily into the solution on the mucosal side and is secondarily transferred to the other side. Added lactate, however, was not found to be transported against a concentration gradient by the hamster intestine (Table 9). Cori & Cori (1928) found that lactate absorption in the rat *in vivo* was much slower than that of glucose and was proportional to the amount fed.

The mechanism by which concentration gradients of lactate and hydrogen ions are developed across the wall of the intestine remains obscure.

The mechanism of glucose absorption. The preferential discharge of lactate in one direction suggests that the formation of lactic acid may play a role in the absorption of glucose. Hestrin-Lerner & Shapiro (1953) have recently reported experiments with rat intestine in which radioactive glucose placed on the mucosal side is converted into an unidentified derivative, most of which appeared on the serosal side. The experiments reported here suggest that most of this derivative is lactic acid. Fisher & Parsons (1953) have shown that three times as much glucose is utilized by the rat intestine with glucose only on the mucosal side than with glucose on the serosal side. It is possible that the increased utilization represents an increased production of lactic acid. If this inference is correct then lactic acid production would increase during absorption of glucose from the lumen of the intestine. It is suggested that this conversion of glucose into lactate may play an important role in the absorption of glucose, especially in the cat and rat in which high rates of aerobic glycolysis have been demonstrated.

It must be emphasized that *in vitro* preparations of both rat (Fisher & Parsons, 1949*a, b*) and hamster intestine (Wilson & Wiseman, 1953*b*) are also capable of transporting glucose as such across the wall against concentration gradients, the hamster intestine transporting 2–5 times as much as that of the rat. A third mechanism for absorption of glucose is passive diffusion in the direction of the concentration gradient. This may be quantitatively unimportant, at least in the guinea pig intestine *in vitro*, since Darlington & Quastel (1953) have shown that with 0.5% glucose on the mucosal side and none on the serosal side the movement of glucose across the wall was inhibited up to 90% by metabolic inhibitors and anaerobic conditions. The relative importance of these three mechanisms (transport of

glucose as such, conversion of glucose into lactate, and passive diffusion of glucose) probably varies considerably with different conditions *in vitro* and *in vivo* and also with different species of animals.

The movement of water and salts across the intestine. The movement of water and salts across the wall of the intestine was regularly observed in the preparations used; in the experiments with everted sacs (Table 5) this movement of fluid was against a slight hydrostatic pressure. There was, however, no evidence of the transfer of sodium or potassium against concentration gradients and no significant difference in the concentration of total ions on the two sides of the intestine was observed (in Table 5 the total concentration of measured ions was 309 m-equiv./l. on the mucosal side and 313 m-equiv./l. on the serosal side). Since the concentration of glucose was 17 mM on the serosal side and 5 mM on the mucosal side, there was probably a difference of osmotic pressure on the two sides. The movement of water and salts was from the side of lower to that of higher osmotic pressure (neglecting the small amounts of protein and undetermined ions on the two sides). In the absence of glucose there was no movement of fluid (Table 6) but this result is not decisive since the tissue may need added glucose as substrate to maintain its normal metabolism. Although there is evidence (Ingraham & Visscher, 1936; Visscher *et al.* 1944) that active processes are involved in the absorption of water and salts *in vivo*, the present *in vitro* experiments may be explained by physical forces.

Secretion of bicarbonate. The movement of bicarbonate across the wall of hamster ileum against a concentration gradient is comparable with that observed with everted frog gastric mucosa secreting hydrochloric acid into bicarbonate solutions (Davies, 1948). In the case of gastric mucosa, bicarbonate also moves across the wall against a concentration gradient, though in the opposite direction from that with hamster ileum.

SUMMARY

1. When glucose-containing saline was circulated through rat intestine according to the method of Fisher & Parsons (1953) the concentration of lactate rose on the serosal side up to 9 times that on the mucosal side within 1 hr. The bicarbonate concentration decreased to about half the original value on the mucosal side while that on the serosal side remained practically constant. Thus glucose is converted into lactic acid, the lactate appearing mainly on the serosal side, the hydrogen ion appearing on the mucosal side.

2. This was confirmed in experiments with sacs of everted intestine of the rat. Concentration gradients of lactate of 6 to 20 were observed. The concentration of bicarbonate decreased on the

mucosal side while that on the serosal side remained constant or increased.

3. Anaerobically the bicarbonate concentrations on both sides of the sacs of rat intestine decreased, the decrease being equivalent to the increase in lactate concentration on the two sides. The lactate gradient did not disappear under anaerobic conditions.

4. Possible mechanisms for the lactate gradients are discussed.

5. It is suggested that the conversion of glucose into lactate may play a role in the absorption of glucose *in vivo*.

6. Sacs of everted ileum from the hamster can transfer bicarbonate from serosal to mucosal sides against a concentration gradient.

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