

BBA 75642

EFFECT OF LUMINAL pH ON THE ABSORPTION OF WATER,  $\text{Na}^+$  AND  $\text{Cl}^-$  BY RAT INTESTINE *IN VIVO*

B. ROUSSEAU\* AND G. E. SLADEN

*Department of Gastroenterology, St. Bartholomew's Hospital, London E.C.1 (England)*

(Received December 14th, 1970)

## SUMMARY

The effects of luminal pH on the net absorption of water,  $\text{Na}^+$  and  $\text{Cl}^-$  from *in vivo* loops of ileum and colon of rat have been studied. Using  $\text{HCO}_3^-$ -containing solutions, absorption of water,  $\text{Na}^+$  and  $\text{Cl}^-$  takes place at an initial pH of 7.6 in the ileum, though it is negligible (water and  $\text{Na}^+$ ), or reduced ( $\text{Cl}^-$ ) at initial pH's of 6.6 and 5.6. In the colon, absorption takes place between pH's 5.6 and 7.6 with an optimum pH of 6.6. At pH 4.2 there is negligible absorption of water and  $\text{Na}^+$  but significant absorption of  $\text{Cl}^-$ .

There is evidence that absorption of water and electrolytes by the small intestine of certain species is inhibited by an acidic luminal environment<sup>1-3</sup>. This phenomenon has, however, received little attention and the most detailed report<sup>1</sup> related only to jejunal absorption. In the present report some effects of luminal pH on net water and electrolyte absorption by the ileum and colon of the rat are described.

Male Albino rats weighing 250–350 g were fasted for 12 h, but allowed access to tap water. Under ether anaesthesia, closed loop experiments were performed on the distal 20–30 cm of ileum or the proximal 5–8 cm of colon, following an initial rinse with saline and air. 5 ml test solution was introduced into each ileal loop and 3 ml into each colon loop. The loops were returned to the abdominal cavity for periods of 30 min (ileum) or 45 min (colon), a temperature of 37° being maintained throughout. At the end of each study the luminal contents were obtained for analysis and the length of the segment recorded.

Six different test solutions were used, covering a pH range of 4.2–7.6. The solutions were all isotonic and contained from 100 mM to 149 mM NaCl. Three solutions had differing concentrations of  $\text{HCO}_3^-$ , viz. 30 mM (pH 7.6), 3 mM (pH 6.6) and 0.3 mM (pH 5.6), and these solutions were gassed with 95 %  $\text{O}_2$ –5 %  $\text{CO}_2$  prior to introduction into the loops. Two solutions contained phosphate, viz. 2 mM  $\text{NaH}_2\text{PO}_4$  plus 14 mM  $\text{Na}_2\text{HPO}_4$  (pH 7.6) and 10 mM  $\text{NaH}_2\text{PO}_4$  plus 7 mM  $\text{Na}_2\text{HPO}_4$  (pH 6.6). The remaining solution contained 23 mM  $\text{Na}_2\text{HPO}_4$  and 18 mM citric acid (pH 4.2). All solutions contained polyethylene glycol in a concentration of 3 g/l. Initial recovery experiments showed that polyethylene glycol was a satisfactory volume marker under these experimental conditions. In six ileal loops the range of polyethylene glycol

\* Present address: Notre-Dame Hospital, 1560 Sherbrooke East, Montreal 133, P.Q. Canada.

recovery, using a rinsing technique, was 96.8–103.3 %. In six colon loops, the range was 96.1–102.1 %.

The initial solutions and samples were analysed for pH using a direct-reading pH meter, for  $\text{Na}^+$  and  $\text{K}^+$  by flame photometry and for  $\text{Cl}^-$  by coulometric titration. Polyethylene glycol was analysed by the turbidometric method of HYDEN<sup>4</sup>. Absorption rates of water and solute were calculated from standard formulae. Wilcoxon's sum of ranks and signed rank tests were used to calculate the significance of differences between and within the various groups<sup>5</sup>.

A summary of the mean absorption rates is found in Table I. In the ileum, appreciable absorption of water,  $\text{Na}^+$  and  $\text{Cl}^-$  took place from the  $\text{HCO}_3^-$ -buffered solution at pH 7.6. However, from solutions at pH 6.6 and 5.6, the absorption rates of water and  $\text{Na}^+$  were not significantly different from zero ( $P > 0.1$ ). Reduction of luminal pH produced a significant reduction of  $\text{Cl}^-$  absorption ( $P < 0.01$ ), but the mean absorption rate was significantly greater than zero at both pH 6.6 and 5.6 ( $P < 0.002$ ). At an initial pH of 7.6 replacement of  $\text{HCO}_3^-$  by phosphate did not significantly affect the mean absorption rates of  $\text{Na}^+$  or  $\text{Cl}^-$  ( $P > 0.1$ ), although the mean water absorption rate was somewhat greater ( $P < 0.05$ ). This suggests that the inhibition of water and  $\text{Na}^+$  absorption at pH 6.6 and 5.6 is not related to the relative lack of luminal  $\text{HCO}_3^-$ , but is probably related to the change of  $\text{H}^+$  concentration. In this respect the jejunum behaves differently in that  $\text{Na}^+$  and water absorption is stimulated by luminal  $\text{HCO}_3^-$  (refs. 6, 7).

In the colon, maximal absorption rates of water,  $\text{Na}^+$  and  $\text{Cl}^-$  took place from the solutions of pH 6.6, irrespective of the presence of  $\text{HCO}_3^-$  or phosphate. These rates were significantly greater than those from solutions of pH 7.6 or 5.6 ( $P < 0.002$ ). Mean absorption rates of water ( $P = 0.05$ ),  $\text{Na}^+$  and  $\text{Cl}^-$  ( $P < 0.002$ ) from the solution of pH 5.6 were significantly greater than zero. Water and  $\text{Na}^+$  absorption was however negligible ( $P > 0.1$ ) from the solution of pH 4.2, although significant  $\text{Cl}^-$  absorption

TABLE I

ABSORPTION RATES OF WATER,  $\text{Na}^+$ ,  $\text{Cl}^-$  AND  $\text{K}^+$  FROM THE ILEUM AND COLON OF RATS

Mean values  $\pm 1$  S.E. (number of rats in each group). Negative sign indicates net entry into the lumen.

| Initial pH    | Buffer             | Water<br>( $\mu\text{l}/\text{min}$ per cm) | $\text{Na}^+$<br>( $\mu\text{equiv}/\text{min}$<br>per cm) | $\text{Cl}^-$<br>( $\mu\text{equiv}/\text{min}$<br>per cm) | $\text{K}^+$<br>( $\mu\text{equiv}/\text{min}$<br>per cm) |
|---------------|--------------------|---|--|--|---|
| <i>Ileum:</i> |                    |   |  |  |   |
| 7.6           | $\text{HCO}_3^-$   | $0.745 \pm 0.051$ (9)                       | $0.119 \pm 0.006$ (9)                                      | $0.152 \pm 0.012$ (9)                                      | $-0.024 \pm 0.001$ (9)                                    |
| 6.6           | $\text{HCO}_3^-$   | $0.021 \pm 0.057$ (9)                       | $-0.003 \pm 0.008$ (9)                                     | $0.075 \pm 0.010$ (9)                                      | $-0.029 \pm 0.002$ (9)                                    |
| 5.6           | $\text{HCO}_3^-$   | $0.018 \pm 0.066$ (6)                       | $0.013 \pm 0.010$ (6)                                      | $0.086 \pm 0.009$ (6)                                      | $-0.017 \pm 0.001$ (6)                                    |
| 7.6           | $\text{PO}_4^{3-}$ | $1.339 \pm 0.205$ (7)                       | $0.107 \pm 0.035$ (7)                                      | $0.184 \pm 0.023$ (7)                                      | $-0.022 \pm 0.001$ (7)                                    |
| <i>Colon:</i> |                    |   |  |  |   |
| 7.6           | $\text{HCO}_3^-$   | $0.888 \pm 0.087$ (6)                       | $0.230 \pm 0.025$ (6)                                      | $0.237 \pm 0.012$ (6)                                      | $-0.025 \pm 0.003$ (6)                                    |
| 6.6           | $\text{HCO}_3^-$   | $1.664 \pm 0.173$ (6)                       | $0.390 \pm 0.017$ (6)                                      | $0.486 \pm 0.022$ (6)                                      | $-0.031 \pm 0.002$ (6)                                    |
| 5.6           | $\text{HCO}_3^-$   | $0.339 \pm 0.131$ (6)                       | $0.130 \pm 0.022$ (6)                                      | $0.215 \pm 0.033$ (6)                                      | $-0.029 \pm 0.004$ (6)                                    |
| 6.6           | $\text{PO}_4^{3-}$ | $1.130 \pm 0.157$ (9)                       | $0.299 \pm 0.030$ (9)                                      | $0.343 \pm 0.033$ (5)                                      | $-0.037 \pm 0.004$ (5)                                    |
| 4.2           | $\text{PO}_4^{3-}$ |   |  |  |   |
|               | citric acid        | $0.374 \pm 0.156$ (9)                       | $0.034 \pm 0.033$ (9)                                      | $0.223 \pm 0.043$ (9)                                      | $-0.025 \pm 0.002$ (9)                                    |

occurred ( $P < 0.002$ ). From all solutions, in the ileum and colon,  $\text{Cl}^-$  absorption was greater than that of  $\text{Na}^+$ , although this was not significant at pH 7.6 ( $P > 0.1$ ). Entry rates of  $\text{K}^+$  into the lumen varied little with luminal pH. Histological examination of the mucosa from many of the rats showed no evidence of a direct injurious effect of low pH, and this is supported by the lack of any demonstrable effect of pH on  $\text{K}^+$  entry into the lumen.

These observations show that reduction of luminal pH does inhibit the net absorption of water,  $\text{Na}^+$  and  $\text{Cl}^-$  from rat ileum and colon. The colon is less sensitive to acid inhibition than the ileum, and appears to have a lower pH optimum for absorption.  $\text{Cl}^-$  absorption is less affected by luminal acidification than water and  $\text{Na}^+$  absorption. The similar effects of pH on water and  $\text{Na}^+$  absorption support the concept that  $\text{Na}^+$  absorption provides the main drive for water movement out of the lumen<sup>8</sup>.  $\text{Cl}^-$  absorption in excess of  $\text{Na}^+$  absorption is probably the result of anion exchange with  $\text{HCO}_3^-$  (ref. 9). This exchange mechanism is relatively insensitive to reduction of luminal pH and can operate in the absence of net movement of  $\text{Na}^+$  or water.

#### ACKNOWLEDGMENTS

The authors wish to thank Dr. A. M. Dawson for much helpful advice and Messrs. P. Hutchinson, P. Bailey and L. Ellam for technical assistance. B. R. was supported by the Medical Research Council of Canada and G. E. S. by the Wellcome Trust.

#### REFERENCES

- 1 G. J. R. MCHARDY AND D. S. PARSONS, *Quart. J. Exptl. Physiol.*, **42** (1957) 33.
- 2 C. F. CODE, P. BASS, J. B. MCCLARY, JR., R. L. NEWNUM AND C. L. ORVIS, *Am. J. Physiol.*, **199** (1960) 281.
- 3 R. HOCHMAN, P. K. KOTTMEIER, R. ADAMSONS AND C. DENNIS, *Am. J. Surg.*, **119** (1970) 64.
- 4 S. HYDEN, *Ann. Swed. Agr. Coll.*, **22** (1955) 139.
- 5 R. LANGLEY, *Practical Statistics*, Pan Books Ltd., London, 1968, p. 166.
- 6 G. E. SLADEN AND A. M. DAWSON, *Nature*, **218** (1968) 267.
- 7 J. S. FORDTRAN, F. C. RECTOR, JR. AND N. W. CARTER, *J. Clin. Invest.*, **47** (1968) 884.
- 8 S. G. SCHULTZ AND P. F. CURRAN, in C. F. CODE, *Handbook of Physiology, Section 6, Alimentary Canal*, Vol. III, American Physiological Society, Washington, D.C., 1st edition, 1968, p. 1245.
- 9 K. A. HUBEL, *Am. J. Physiol.*, **213** (1967) 1409.