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TRANSPORT OF SODIUM AND SECRETION OF POTASSIUM AND BICARBONATE BY THE COLON OF NORMAL AND SODIUM-DEPLETED RATS

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SUMMARY

1. Ascending and descending colonic segments of normal and Na-depleted rats were perfused *in vivo* with isotonic solutions of varying Na concentration and the unidirectional Na fluxes and secretion rate of K and bicarbonate and the transmucosal electrical p.d. were measured.

2. Potential difference was greater in Na-depleted rats, especially towards the distal end of the descending colon. With reduction of luminal Na concentration, p.d. was reduced.

3. The ascending and descending segments were similar in regard to Na transport except that the latter had lower passive permeability. Na depletion caused an increase of Na influx rate, Na net flux rate and Na exchange diffusion whilst the mucosal passive Na permeability decreased. These changes resulted in a reduction in the critical luminal Na concentration, i.e. the concentration at which the unidirectional fluxes were equal.

4. K secretion rate was similar in the ascending and descending colon and was increased by Na depletion. In all rats, it was reduced when the luminal Na concentration was low.

5. Bicarbonate secretion rate was unaffected by the Na depletion and all solutions remained isotonic during perfusion.

6. The results confirmed that active Na transport was stimulated by Na depletion but indicated that this was probably not the only factor in the elevation of transmucosal p.d.

INTRODUCTION

It was shown in the previous paper (Edmonds, 1967*a*) that Na depletion resulted in a fall in the Na content of rat faeces removed from various parts of the colon, a rise in K content and an elevation of the electrical p.d.

between the luminal and serosal surfaces of the colon, and it was concluded that Na depletion stimulated Na absorption. The present paper reports experiments which confirm this finding, and analyses in more detail the effect of Na depletion on Na transport across the colon wall and on K and bicarbonate secretion. The ascending and descending segments of colon were examined independently since their electrical response to Na depletion differed.

METHODS

Male albino rats weighing 300–350 g were prepared as described previously (Edmonds, 1967*a*). When the rats were Na-depleted, at least 7 days were allowed before experiment and all rats were fed on a rice diet for 7–10 days before use.

For the perfusion experiments the rats were anaesthetized by intraperitoneal injection of Nembutal, 6 mg/100 g body wt. Nembutal was injected into the lower abdomen in the mid line to avoid its being directly injected into the region of the colon. Several experiments carried out initially showed that whether anaesthesia was induced by this method or by open ether or by intravenous Nembutal, there was no significant difference in the colonic p.d. Furthermore, it was found that even when a little Nembutal at the above concentration was applied directly to the serosal surface of the colon, the p.d. was unaffected. With the rat anaesthetized, the abdomen was opened by mid-line incision. The colon was washed out with warm saline and the ascending and descending segments cannulated separately, the distal cannula in both cases communicating with a small reservoir from which samples could be taken. The reservoirs and the proximal cannulae were connected by polythene tubes to a peristaltic pump which recirculated fluid continuously during the experiments at a rate of 2·5 ml./min. The fluid passed from the pump through a small coil immersed in a water-bath at 38° C before re-entering the bowel. The body temperature of the rat was maintained constant by a heating pad placed beneath it, the temperature of the pad being controlled by a transistor inserted into the rectum (Krnjević & Mitchell, 1961). Ionic fluxes were measured using NaCl solutions of concentration of 150 mM, 75 mM and 25 mM, the latter solutions being made isotonic with 150 mM-NaCl by the addition of mannitol. For the flux measurements, two perfusions were carried out with each solution for 30 min. The solutions contained ^{24}NaCl (0·3 mμc/μmole) for the measurement of outflux and phenol red as an indicator of water movements, trial experiments having confirmed that there was no significant absorption of this dye.

The standard procedure was initially to wash the gut and the circulating system with a solution of appropriate concentration but without phenol red or ^{24}Na. As much as possible of this was then removed and a known volume of perfusion solution introduced. The fluid was recirculated for 5 min to allow complete mixing, and an initial sample then taken. Circulation was continued for a further 30 min, when the final sample was taken. From the initial phenol-red concentration the volume circulating at the beginning of the perfusion period was calculated and from the final phenol-red concentration the water absorption could be determined. The volume circulated ranged from 2·8 to 3·8 ml. At the end of each experiment the colon was removed and a glass tube (o.d. 7 mm for the ascending colon and 6 mm for the descending colon) was passed through the lumen, the gut being pulled on-to the tube, so that it was fully expanded but not stretched, and the length measured. The muscle coat was stripped off and the dry weight of the mucosa determined after 24 hr in an oven at 98° C. The K content was subsequently estimated after digestion for 8 hr in concentrated nitric acid on a hot plate. Mucus was often present in the perfusate at the end of the circulation. In the majority of specimens the amount of mucus was small, but in the earlier perfusions of an experiment and particularly in specimens coming from the ascending colon, appreciable quantities of mucus were sometimes present. Where this was observed,

the specimens were centrifuged before samples were taken for analysis. As the K content of the perfusates might in part have been attributable to desquamated cells or cellular debris, some specimens were ultracentrifuged to remove such material. This procedure did not however significantly affect the K content, so presumably any contribution by cellular debris to the K content of the perfusate was small.

P.d. measurements. The use of 3M-KCl-agar 4% bridges was not satisfactory since it was found that significant contamination of the luminal solutions with K could occur whilst the p.d. measurements were being made. A set of agar bridges was therefore prepared using the same solution as were perfused and the appropriate bridge was placed in the lumen according to the NaCl solution circulated. Thus the junction potential which otherwise would be present from contact of solutions of differing NaCl concentration was eliminated. These electrodes were marked at 1 cm intervals so that the position of the tip within the lumen was known and they were introduced through the cannulae which fed the reservoir The serosal side electrode was placed in the peritoneal cavity, electrical contact being made by introducing a little 150 mM-NaCl. This electrode had a composition, 150 mM-NaCl-agar 4%. The bridges were connected through saturated KCl wells and calomel half-cells to a 'Vibron' electrometer (Model 33B, Electronic Instruments Ltd.). The asymmetry potential of the circuit was checked at intervals during the experiments and was not acceptable i greater than 1 mV, the observed value being subtracted from those measured. To check the asymmetry potential a U-tube was employed containing 3 mM-KCl-agar 4% but with capacity for about 5 ml. of a solution in each arm. Into one side was placed 150 mM-NaCl and into this solution the serosal side bridge was introduced. Into the other arm was placed a solution of the same composition as the perfusion solution being circulated and into it was put the bridge being employed in the luminal solution. This method eliminated junction potentials due to contact of different NaCl solutions.

Tracer and chemical measurements. ^{24}Na was determined in a well-type NaI crystal scintillation counter to an accuracy of $\pm 1{\cdot}5\%$ (this and subsequent accuracies are expressed as ± 1 S.D. based on 10 replicates). Phenol red concentration was determined by adding 0·1 ml. of test solution to 3 ml. of 0·1 M-NaOH and measuring the optical density at 550 mμ to an accuracy of $\pm 2{\cdot}6\%$. Na and K were determined by an EEL flame photometer. The accuracy of Na determinations over the range examined was $\pm 1{\cdot}5\%$ but for K was $\pm 2{\cdot}3\%$ as the solutions had very low concentrations. In determinations on solutions containing little K relative to Na, K standards were used containing appropriate amounts of Na. Bicarbonate was measured by Conway's diffusion method to an accuracy of $\pm 2{\cdot}6\%$ (Conway, 1957) and osmolality with a Fiske osmometer.

Terminology and calculations. Since several schemes have been used in describing ionic fluxes in the gut, some consideration has to be given to the definition of the terms used here. Authors who have employed methods involving the placing of an isotope in the lumen and observing changes in luminal fluid composition have favoured terms influenced by the method (e.g. Visscher, Varco, Carr, Dean & Erickson, 1944; Curran & Solomon, 1957). Berger, Kanzaki & Steele (1960), for example, defined 'undirectional flux' in the generally accepted manner as the one-way passage of electrolyte from one side of the intestinal mucosa to the other, but proceeded to use the term 'influx' as denoting flow into and 'efflux' as flow out of the intestinal lumen. Thus, the terms were transferred from isotope fluxes to ionic fluxes, ^{24}Na efflux, for example, becoming Na efflux. This is confusing, however, because isotope may be placed in the animal so that efflux then has the reverse sense. It is preferable therefore to use the terms in relation to ionic flux with the animal as the reference rather than in a way dependent on where the isotope is placed. Code (1960) employed a scheme of this sort which has since been adopted by others for isotopic studies in man (e.g. Duthie & Atwell, 1963). He used the term 'insorption' to describe the unidirectional flux from lumen to blood and 'exsorption' for that from blood to lumen, the ionic fluxes thus being described as flows into and out of the animal.

The system adopted in the present paper is developed from that of Code and considers flow as into and out of the animal irrespective of where the tracer is placed. 'Unidirectional flux' is defined as stated above. 'Influx' is defined as flow from lumen to plasma and 'efflux' as flow from plasma to the lumen (these definitions are the reverse of those of Berger *et al.* 1960). The terms 'absorption' denoting a net decrease and 'secretion' a net increase in luminal content of water or electrolyte are used in the customary sense and these definitions are similar to those of Berger *et al.* (1960). The conventional symbol for flux rate, J, is employed, J_i representing influx, J_o efflux, and J_n net flux. Some difficulty arises when we attempt to combine influx and efflux to obtain net flux. Curran & Solomon (1957), for example, state the relationship $J_n = J_o - J_i$, which if $J_i > J_o$ results in the net flow being less than zero, an apparent absurdity arising because the sign of J refers to the direction of flow. Hence we have also to adopt a sign convention to indicate this direction. Again taking the animal as the reference, we assign to flow out of the animal a negative sign, i.e. J_o is negative, and to flow into the animal a positive sign, i.e. J_i is positive. Then net flux is given by $J_n = J_i + J_o$ and its sign will indicate its direction. With secretion, J_n is negative and with absorption, positive. When the luminal concentration of an ion is such that J_n is zero, this concentration is called in the present paper 'the critical luminal concentration'.

The calculation of fluxes was based on a two-compartment model, the lumen being considered as one compartment and the body fluids as the other. The equations for such a system applied to rat gut have been derived by Curran and his associates (Curran & Solomon, 1957; Curran & Schwartz, 1960) and they have confirmed the validity of the model. Results are given as means ± 1 S.E. of the mean.

RESULTS

P.d. experiments

In preliminary experiments the stability of the p.d. was observed. Continuous recording of the p.d. over several hours showed that there was little variation. Small wave-like changes were often seen but they were of low amplitude, rarely exceeding 2 mV or lasting more than 2 min. In view of this stability the p.d. was measured on three occasions only during the perfusion—at the beginning, midpoint and just before the end—and the mean obtained.

TABLE 1. Electrical p.d. measurements made at the proximal and distal ends of segments of ascending and descending colon of six normal and six Na depleted rats during perfusion of various NaCl solutions. Results are given as mean ± 1 S.E.

NaCl concentration (mM)...	Ascending			Descending		
	150	75	25	150	75	25
			Control			
Proximal	5·9 ± 0·6	4·2 ± 0·4	2·6 ± 0·4	6·2 ± 0·6	4·4 ± 0·6	2·1 ± 0·3
Distal	6·1 ± 0·5	3·8 ± 0·3	1·6 ± 0·2	8·4 ± 0·8	6·6 ± 0·8	1·8 ± 0·3
			Na-depleted			
Proximal	12·5 ± 0·5	5·6 ± 0·4	4·1 ± 0·3	16·6 ± 0·8	10·2 ± 0·8	10·1 ± 0·7
Distal	9·7 ± 0·6	4·2 ± 0·4	2·5 ± 0·5	37 ± 3·0	37 ± 3·0	32 ± 3·1

In the control rats with 150 mM-NaCl in the lumen the p.d. was similar in the ascending and descending segments and showed little variation

along the length of the colon (Table 1, Fig. 1). In the Na-depleted rats the potentials were higher than in the controls and there was also a striking difference between the ascending and descending segments, the former showing little variation along the length of the gut while in the descending colon there was a rapid rise of potential as the electrode in the lumen was advanced from the proximal to the distal end.

When 75 mM and 25 mM-NaCl solutions were perfused the large p.d. seen in the descending colon of the Na-depleted rats changed little, but the lower potentials both in the control and Na-depleted rats tended to fall and occasionally even reversed so that the lumen became positive with respect to the serosal surface.

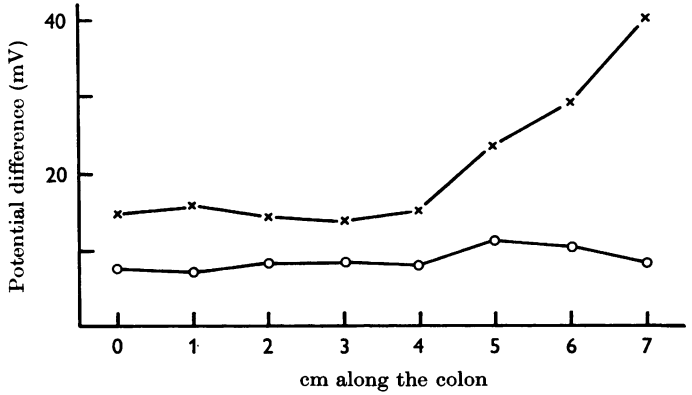

Fig. 1. Electrical p.d. measurements made in one of the normal and one of the Na-depleted rats. Measurements were made at 1 cm intervals along the ascending (0–4 cm) and descending (4–7 cm) colon, when lumen contained 150 mM-NaCl solution. ×, Na depleted rat; ○, normal rat.

Ionic fluxes

The flux measurements in this paper were related to the length of colon as measured on standard glass tubes. As the rats were of similar size, there was little variation of colon diameter so that surface area varied mainly according to length. It was found also that the dry weight of the mucosa and the K content bore a similar relationship to length both in the ascending and descending colon and irrespective of whether the rats were Na-depleted or in normal condition (Table 2). Surface area could be calculated from the gut length and the diameter of the glass tube which was used to measure length. Calculated in this way, the ascending colon had a surface area of 2·2 cm^2/cm colon length and the descending colon 1·9 cm^2/cm length.

Na influx. In the control rats, Na influx rate was similar in the ascending and descending segments when 150 mM-NaCl was perfused (Fig. 2) and the

same was true when 75 mM-NaCl was perfused. With 25 mM-NaCl, however, the influx rate was less in the descending than in the ascending colon ($P < 0.05$). Na depletion led to an increase in Na influx rate in the ascending colon when 150 mM-NaCl was perfused ($P < 0.05$) but with 75 mM and 25 mM-NaCl no significant effect was observed. In the descending colon, however, Na depletion resulted in an increased influx rate with all NaCl concentrations studied ($P < 0.005$). When the ascending and descending segments of the Na-depleted rats were compared, no significant difference in Na influx rate between the segments was found when 150 mM or 75 mM-NaCl were perfused, but when 25 mM-NaCl was used the influx rate in the descending was greater than in the ascending colon ($P < 0.01$).

TABLE 2. Length of gut used in perfusion experiments and relationship of mucosal dry weight to the K content in ascending and descending segments of colon of six normal and six Na-depleted rats. Results are given as mean with range or as mean ± 1 S.E.

	Colon segment	Length of colon perfused (cm)	Dry weight per unit length (mg/cm)	K per unit length (μ mole/cm)
Control	Ascending	3·9 (3·2–5·0)	15·9 ± 1·8	6·2 ± 0·7
	Descending	2·9 (2·1 – 3·5)	17·2 ± 2·0	6·3 ± 0·9
Na-depleted	Ascending	4·3 (3·5 – 1·7)	15·5 ± 1·9	6·2 ± 0·9
	Descending	3·0 (2·0 – 3·9)	15·3 ± 1·1	6·4 ± 0·5

Na efflux. Na efflux rate declined in all instances when the luminal NaCl concentration was reduced. Comparison of Na efflux rate when 150 mM-NaCl was perfused with that when 25 mM-NaCl was perfused showed that in the control rats the efflux rate with 150 mM-NaCl was significantly higher both for the ascending ($P = 0.05$) and descending colon ($P < 0.05$). The effect was even more evident after Na depletion; the differences in both the ascending ($P < 0.005$) and descending colon ($P < 0.005$) being highly significant.

Na efflux rates of the ascending colon were compared with those of the descending colon at each NaCl concentration perfused. In the controls, it was at a higher rate in the ascending colon with all NaCl concentrations ($P < 0.005$). In the Na-depleted rats, however, no significant differences were observed at any of the concentrations examined.

An estimate of the rate of passive diffusion of Na from the extracellular fluid (e.c.f.) to the lumen could be made by producing the efflux lines back to the vertical axis where the luminal Na concentration would be zero. Circulation of NaCl-free solutions was not, however, carried out in these experiments, so there must be some reservation as to the complete

validity of the extrapolation. In the control rats, Na influx rate at zero luminal NaCl concentration obtained by extrapolation was 285 n-mole/min/cm for the ascending colon and 125 n-mole/min/cm for the descending

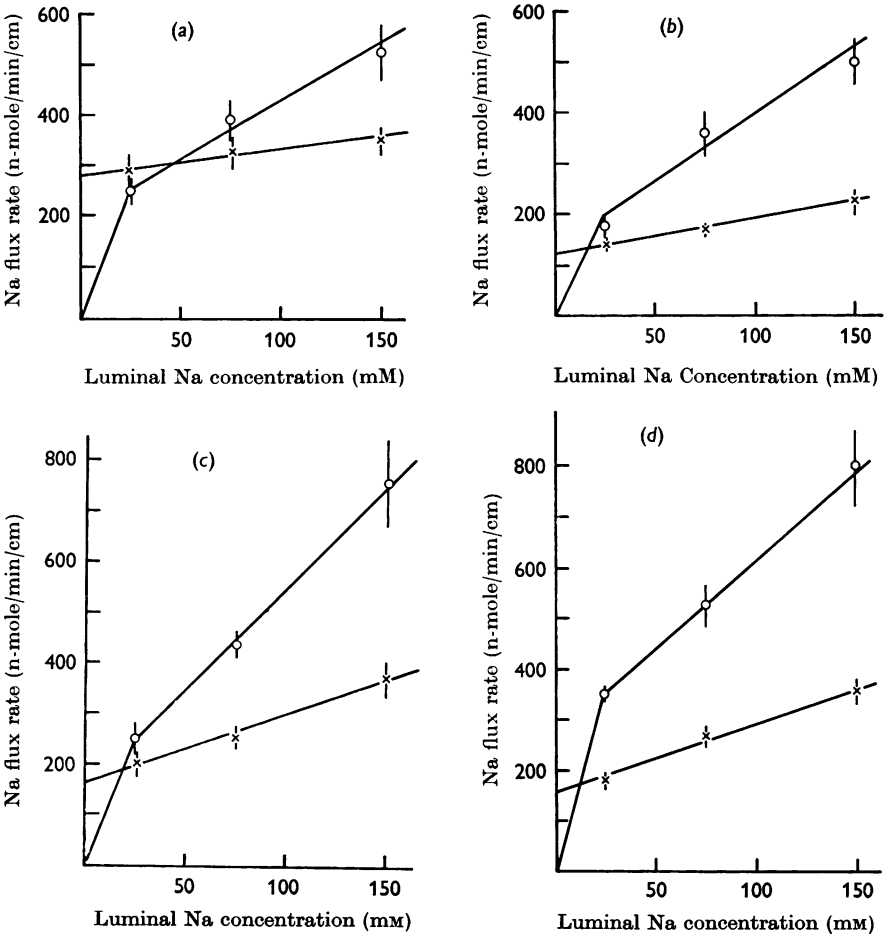

Fig. 2. Influx and efflux rates of Na measured with various concentrations of NaCl solution in the lumen in six normal and six Na-depleted rats. ○, Na influx; ×, Na efflux. Results are given as a mean ±1 S.E. *a*, Ascending colon of normal rats; *b*, descending colon of normal rats; *c*, ascending colon of Na-depleted rats; *d*, descending colon of Na-depleted rats.

colon. The striking difference suggests that the descending colon has about half the permeability to the passive flow of Na ions as has the ascending colon. In the Na-depleted rats the Na efflux rate for zero luminal NaCl was 164 n-mole/min/cm in the ascending colon and 155 n-mole/min/cm in the descending colon. The slopes of the Na efflux lines were similar for each

segment but appreciably steeper than in the control state. The passive permeability coefficient (P_{Na}) for each colonic segment was calculated from these values on the assumption that Na efflux was entirely due to simple diffusion when the luminal Na concentration was zero, the effect of p.d. being for the moment ignored. For the ascending colon, P_{Na} was $2{\cdot}0 \times 10^{-3}$ cm³/min/cm gut length in the control rats and $1{\cdot}2 \times 10^{-3}$ cm³/min/cm in the Na-depleted. The former value is close to that obtained by Curran & Schwartz (1960) in their normal rats. In the descending colon, P_{Na} was $0{\cdot}9 \times 10^{-3}$ cm³/min/cm in the controls and $1{\cdot}1 \times 10^{-3}$ cm³/min/cm in the Na-depleted rats. The effect of p.d. was not considered in these calculations and it would be relatively small where the p.d. was low as in the normal animals. Where, however, the p.d. was considerable as in the descending colon of the Na-depleted rats a quite large overestimate of P_{Na} was made. It seems likely therefore that in both segments of colon the passive permeability was decreased by Na depletion.

TABLE 3. Net flux rate of Na in ascending and descending colon of six normal and six Na-depleted rats during perfusion of various NaCl solutions. Results are given as mean ± 1 S.E.

	Na net flux rate (n-mole/min/cm)					
	Ascending			Descending		
NaCl concentration (mM) ...	150	75	25	150	75	25
Control	227 ± 18	65 ± 6	−32 ± 11	273 ± 20	189 ± 15	28 ± 14
Na-depleted	383 ± 21	184 ± 16	49 ± 9	470 ± 39	262 ± 22	171 ± 15

Na net flux. Na net flux was greater in the descending than ascending colon in the control and in the Na-depleted rats with all solutions (Table 3). When the 150 mM-NaCl was in the lumen, the difference between the ascending colon and descending colon was only just significant at $P < 0{\cdot}05$ but with the 75 mM and 25 mM-NaCl the differences were larger and more significant ($P < 0{\cdot}005$). Na depletion increased the net flux in both the ascending and descending colon at all concentrations used ($P < 0{\cdot}01$). Consequently the critical luminal Na concentration in the ascending colon was displaced by Na depletion from about 50 mM to a little less than 25 mM. In the descending colon the critical luminal Na concentration was lower in the control condition, being about 25 mM, while after Na depletion it was further reduced. In the latter case, there was still a net transfer of Na from lumen even when 25 mM-NaCl was perfused so that the point of balance of the unidirectional fluxes could only be obtained by extrapolation.

On this account, in three Na-depleted rats recirculation of 25 mM-NaCl was carried out for 3 hr to determine the level to which the Na concentration of the circulating fluid was reduced by a descending colon

stimulated by Na depletion. The Na concentration reached at the end of this time was $11 \pm 3{\cdot}1$ mM. The osmolality of the fluid was unchanged by the perfusion. The results of a typical experiment are shown in Fig. 3. Most of the change in Na concentration occurred in the first 2 hr, there being little change subsequently. Water absorption took place throughout so that the unidirectional Na fluxes were never exactly equal. They were, however, very nearly so during the last hour, the net efflux of Na being then only about 5 n-mole/min/cm.

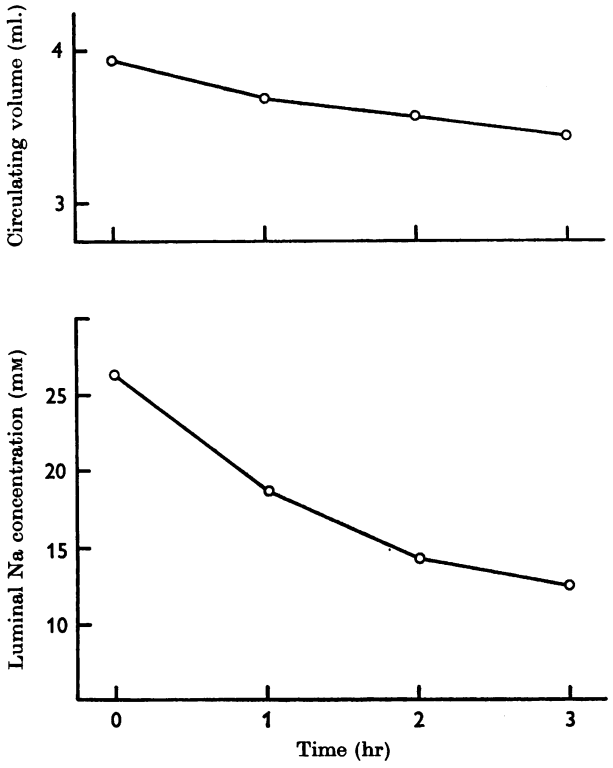

Fig. 3. Change of Na concentration and circulating volume in a Na-depleted rat during prolonged perfusion of the descending colon.

K secretion. During perfusion of NaCl solutions through segments of colon, the rate of increase in luminal K concentration is practically linear over short periods of time (Edmonds, 1967*b*) so that the K secretion rate can be calculated by dividing the amount of K that has appeared in the lumen during the perfusion period by the time of the perfusion.

In the control state, no significant difference was found between the ascending and descending colon in the rate of K secretion (Table 4). Reduction of NaCl concentration in the lumen from 150 to 75 mM had no

significant effect but in both ascending and descending segments there was significantly less K secreted when the 25 mM-NaCl was perfused ($P < 0{\cdot}05$). In Na-depleted rats the K secretion rate was considerably greater than in the control state in both segments of the gut and with all the solutions used. The effect of lowering the NaCl concentration was similar to that observed in the controls, there being significantly less K secretion when 25 mM was compared with 150 mM ($P < 0{\cdot}05$). A further observation was that K secretion rate in the descending colon was higher than in the ascending colon after Na depletion ($P < 0{\cdot}05$).

TABLE 4. Secretion rate of K by the ascending and descending colon of six normal and six Na-depleted rats during perfusion of various NaCl solutions. Results are given as mean ± 1 S.E.

	K secretion rate (n-mole/min/cm)					
	Ascending			Descending		
NaCl concentration (mM) ...	150	75	25	150	75	25
Control	39 ± 3	40 ± 3	34 ± 2	37 ± 4	43 ± 4	30 ± 3
Na-depleted	81 ± 7	76 ± 3	66 ± 6	90 ± 7	95 ± 8	78 ± 6

TABLE 5. Secretion rate of bicarbonate by the ascending and descending colon of six normal and six Na-depleted rats during perfusion of various NaCl solutions. Results are given as mean ± 1 S.E.

	Bicarbonate secretion rate (n-mole/min/cm)					
	Ascending			Descending		
NaCl concentration (mM) ...	150	75	25	150	75	25
Control	75 ± 12	57 ± 7	58 ± 10	71 ± 8	68 ± 8	61 ± 7
Na-depleted	57 ± 7	60 ± 6	52 ± 8	67 ± 5	63 ± 6	60 ± 10

Bicarbonate secretion. The solutions became alkaline during perfusion and this was associated with a rise of CO_2 content (Table 5). As the pH of the solutions did not rise above 8·2, this CO_2 must have been almost entirely present as bicarbonate ion, representing either secretion of bicarbonate ions into the lumen of the gut or the removal of hydrogen ions from the perfusate. The method does not distinguish between the two possibilities but here it is assumed that bicarbonate ions are secreted. The results show that the ascending and descending colons secreted bicarbonate to a similar extent and that the secretion rate was not influenced by Na depletion.

Osmolality. There were no significant changes in osmolality during the 30 min perfusions and, in the case of 3 hr perfusion as mentioned earlier, the osmolality was unchanged despite considerable fall in Na concentration.

DISCUSSION

Sodium. The results on normal rats were like those obtained by Curran & Schwartz (1960). The unidirectional Na fluxes observed in the present experiments were similar in magnitude but the critical Na concentrations were somewhat less, being about 40 mM in the ascending and 25 mM in the descending colon compared with their value of 75 mM found in segments of colon which probably included both ascending and descending colon. The lower concentrations observed here were more in accord with the Na concentrations in the water of the gut contents of our rats (Edmonds, 1967*a*).

Influx of Na fell when the luminal Na concentration was reduced but so too did efflux, indicating that Na movement from plasma to lumen was not accounted for entirely by simple diffusion. The dependence of a Na flux on the Na concentration present in the compartment into which the flux is directed has been observed in several tissues (e.g. in frog skin by Kirschner, 1955, and in red blood cells by Hoffman, 1962) and has been attributed to exchange diffusion. The absorption of Na against the electrochemical gradient is due to active Na transport (Curran & Schwartz, 1960). Thus the present experiments on normal rats confirmed the findings of Curran & Schwartz in indicating that Na transport across the colonic epithelium involved three processes—active transport, passive diffusion and exchange diffusion—but added the information that whilst both segments of colon transported Na actively and to a similar extent, they appeared to differ in passive permeability to Na ions, the descending being substantially less permeable than the ascending colon.

There is little information on the effect of Na depletion on Na fluxes. Ross & Spencer (1954) made an indirect study when observing rates of Na transfer on and off a resin placed in the gut lumen. They were unable to show that restricting Na intake affected the rates. Stool Na was however reduced by Na restriction and they suggested that the essential modification was that Na removal from the resin was possible against a considerably greater chemical gradient when rats were Na depleted than when they were fed a normal diet. The present experiments agreed with previous findings (Edmonds, 1967*a*) in that Na absorption was stimulated by Na depletion, and showed in addition that the kinetics were modified in several ways. The increased Na absorption resulted chiefly from a rise of Na influx which, since Na efflux was either unchanged or reduced, must have been due to increased active Na transport. Further, the exchange diffusion component appeared to be increased, for the steepness of the slopes relating Na influx to luminal Na concentration was greater after Na depletion. Finally, passive permeability to Na appeared to decrease with

Na depletion. The combined result of these effects was to increase the rate of Na absorption and to reduce the critical luminal Na concentration.

The results obtained from prolonged perfusion of the descending colon of Na-depleted rats were consistent with those obtained from analyses of stools and gut contents (Edmonds, 1967*a*) in indicating that the colonic mucosa could absorb Na against a considerable electrochemical gradient. The colonic mucosa compares well with other Na transporting epithelia in this respect (Diamond, 1962).

Potassium and bicarbonate. Ross & Spencer (1954) in experiments with an ion exchange resin were unable to find any evidence for increased K transfer rates during Na restriction, a conclusion in contrast to the present findings. The inconsistency may be due to technique or to differing degrees of Na depletion. It was not sure whether K secretion could be accounted for by simple diffusion from the e.c.f. along the electrochemical gradient. Certainly, after Na depletion the gradient was increased as a result of the rise of the p.d., but although this effect was greater in the descending colon, K secretion was increased to a similar extent in each segment. Furthermore, reducing the luminal Na concentration decreased the K secretion rate although the changes in p.d. were slight. These findings lend support to the suggestion of the previous paper (Edmonds, 1967*a*) that simple diffusion alone was not adequate to account for K secretion but conclusive evidence will be presented subsequently (Edmonds, 1967*b*).

The secretion of bicarbonate ions by the colon has been demonstrated in several species (Parsons, Powell & Pyrah, 1952; D'Agostino, Leadbetter & Schwartz, 1953; Parsons, 1956) and some evidence for active transport of the ion was produced by Cooperstein & Brockman (1958). The present experiments did not allow assessment of the latter possibility but did demonstrate that none of the variations produced by Na depletion affected the secretion rate. It was not dependent on the p.d. across the gut wall, a factor which would be expected to exert an effect if the secretion were simply a result of passive diffusion. Nor was there dependence on the segment of colon used nor the concentration of Na or Cl in the lumen over the range 25–150 mM. This independence is consistent with the hypothesis that the bicarbonate ions are produced within the mucosal cells, possibly by a carbonic anhydrase mechanism followed by diffusion into the lumen (Madson, 1964).

Potential differences. The electrical p.d. across normal rat colonic mucosa was attributed by Curran & Schwartz (1960) to the active transport of Na ions with passive diffusion of Cl. Other ions also cross the colonic mucosa; K and bicarbonate, for example, were transported in the present experiments. The quantity of these ions relative to Na, particularly when the 150 mM-NaCl was in lumen, was small and so they contributed little to the

p.d. It is likely therefore that the p.d. across the mucosa is predominantly a reflexion of active Na transport and that the increase in the latter is responsible for the elevated p.d. In the frog skin (Ussing & Zerahn, 1951), toad bladder (Leaf, Anderson & Page, 1958) and rat proximal renal tubule (Windhager & Giebisch, 1961) the p.d. across the tissue has been described in terms of a Na current due to active Na transport and an ohmic resistance provided by the tissue. If a similar simple model were applicable to the colon, then the alteration in p.d. would reflect either an increased Na current or a decrease in ionic permeability of the tissue producing an increase in its electrical resistance. In the present experiments, with 150 mM-NaCl solution in the lumen, the p.d. approximately doubled in the ascending colon with Na depletion and the Na absorption was nearly doubled. In the descending colon, however, the rise of p.d. was greater, rising three- to four-fold on Na depletion, yet the Na absorption was again less than doubled. One possible explanation is that an alteration in membrane resistance occurred following Na depletion and there was some support for this in the observation that Na passive permeability was reduced by Na depletion.

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