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THE GRADIENT OF ELECTRICAL POTENTIAL DIFFERENCE AND OF SODIUM AND POTASSIUM OF THE GUT CONTENTS ALONG THE CAECUM AND COLON OF NORMAL AND SODIUM-DEPLETED RATS

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SUMMARY

1. The Na, K and water content of stools, and of gut contents removed from the terminal ileum, caecum and colon were determined in normal and Na-depleted rats and the p.d. across the colon wall measured at the site of removal of each specimen.

2. During passage through the caecum and colon, especially the ascending segment of colon, the faecal Na and water content fell considerably, K content being unchanged in the normal rats and falling in the Na-depleted. Na concentration of the faecal water fell but K concentration rose owing to water absorption.

3. Feeding normal rats with a sulphonated polystyrene resin caused a considerable Na loss in the stool, the ratio Na/(Na + K) being consistently greater than in rats not taking resin. Resin induced little Na but much K loss in Na-depleted rats.

4. The electrical p.d. across the colon wall varied little over the length of the caecum and colon in normal rats, rarely exceeding 20 mV, the serosa being +ve with respect to lumen. Potential difference measurements were greater in Na-depleted rats, and those of the caecum and descending colon were consistently higher than those of the ascending colon. There was a similar pattern in resin-fed rats but potentials tended to be higher.

5. K concentration of the gut contents was always greater than could be accounted for if K were passively distributed across the colonic mucosa.

6. It was concluded that: (i) active Na absorption was stimulated by Na depletion; (ii) K was probably actively transported into the colon lumen, and when unabsorbable anions were present in the gut K secretion was critically important in Na absorption; (iii) the elevation of p.d.

associated with Na depletion was probably associated with the stimulated Na transport.

INTRODUCTION

The gut and kidney show a striking parallel in their behaviour when animals or human subjects are stimulated to Na conservation either by Na depletion or by certain pathological processes. When an attempt is made to increase renal Na excretion by administering diuretic drugs, one of the thiazide group, for example, to patients with hepatic cirrhosis and ascites or with cardiac failure or nephrosis (Edmonds, 1960*b*; Shaldon, McLaren & Sherlock, 1960) or to normal men depleted of Na (Edmonds & Wilson, 1960), instead of the customary Na and Cl diuresis characteristic of the normal condition, a considerable renal loss of K and Cl ions with relatively little Na is often produced. In the gut a similar phenomenon is seen if cation exchange resins of a type which normally promote faecal Na excretion are given. In the above diseases and in the Na-depleted state, these resins promote a loss of K rather than Na (Gabuzda, Phillips & Davidson, 1952; Greenman, Shaler & Danowski, 1953; Ross & Spencer, 1954), suggesting that some modification in the handling of Na and K by the colon occurs in Na depletion although little direct evidence has been produced supporting this possibility.

A small electrical potential difference (p.d.) is present across the bowel wall, the serosal surface being positive with respect to the luminal and it has been suggested that this p.d. is due to active Na transport (Curran & Schwartz, 1960; Cooperstein & Hogben, 1959). The present investigation was stimulated by the possibility that an alteration in Na absorption might be reflected in a change in the p.d. Such a change would also have importance in influencing the movement of other ions. The present paper shows that electrical activity of the colon is considerably altered by Na depletion, and evidence from parallel studies on composition of faeces from various parts of the colon suggests that active colonic absorption of Na and secretion of K are modified in this condition.

METHODS

Male albino rats weighing 300–350 g were used. For 7–10 days before experiment they were fed boiled rice in proportions of 100 g dry rice to 200 ml. of distilled water with the addition of 3 m-moles KCl/100 g dry rice. Control rats received 0·5 % NaCl solution as drinking fluid, the Na-depleted group were given tap water.

Na depletion was induced by injecting into the peritoneal cavity 5 ml./100 g body wt. of 5 % (w/v) glucose solution, followed by removal 2 hr later. On average about 0·4 m-moles Na/100 g. body wt. was removed, representing about 8 % of a rat's 24 hr exchangeable Na (Edmonds, 1960*a*). The Na-depleted rats ate little during the first 24 hr after depletion but by 36 hr had recovered fully and appeared healthy at the time of experiment 7–10 days

later, although they still tended to eat somewhat less than the control animals. The latter consumed about 80–100 g of boiled rice daily while for the Na-depleted the amount was 70–80 g. On this diet, the 24 hr intake of Na was about 1·3 m-moles and of potassium 0·7–1·0 m-moles in the control rats; in the Na-depleted rats the K intake was a little less and the Na intake almost zero. The control rats gained weight normally but the Na-depleted rats did not gain during the period of observation before experiment.

In some control animals, Na depletion was induced and animals were subsequently repleted with saline 0·5 % as drinking fluid. These rats were indistinguishable in experiments from untreated controls and accordingly the control rats were not in general subjected to this procedure.

Some control and Na-depleted rats were also studied while they were receiving NH_4 and L polystyrene sulphonate resin ('Katonium'—Bayer Products Ltd.). The resin used in these experiments had a selectivity $K_K^{Na} = 0{\cdot}81$ and a capacity of 2·8 m-mole/g. It was thoroughly mixed into the food so that each rat took about 0·4–0·5 g daily. The resin diet was commenced 3 days before experiments.

It was found that gentle handling of the rats would usually provoke defaecation and the fresh stools were collected in this way. Where total daily stool output was required, the rats were kept in individual metabolism cages. Each cage was placed on a funnel containing a grill which retained stools while allowing urine to pass through. In these experiments, the stools of each group were pooled and after drying were powdered and a weighed aliquot taken for analysis.

Operative procedures. Nembutal 6 mg/100 g body wt. intraperitoneal was used. In some preliminary experiments open ether anaesthesia was used; the results were similar to those obtained with Nembutal and the latter was employed in all the reported experiments.

The abdomen was opened by mid-line incision. By gently rearranging the abdominal contents it was possible to expose segments of the caecum and colon in turn without disturbance of the blood supply. Whilst the electrical potentials were being measured, the surface of the gut was kept moist with warm isotonic saline. The potentials were measured wherever faeces were present and in a few cases at empty sites. On completion of the measurements, the bowel wall was opened using cutting cautery and the gut contents removed for analysis. Blood was taken into heparinized tubes from the internal carotid artery at the end of the experiment.

Measurements of electrical potential. Micro-electrodes were drawn from Pyrex glass tubing (o.d. 2 mm) using a puller of the Alexander & Nastuk type, and filled with 3 M-KCl. Electrodes with a short tapered shank and tip diameter of about 10 μ were used. Electrodes of this type were strong enough to penetrate the bowel wall without breaking. The electrode was inserted into a Perspex holder filled with 3 M-KCl—4 % agar and the holder fixed rigidly to a Prior micromanipulator. The reference electrode consisted of a polythene tube (o.d. 1 mm) filled with 3 M-KCl—4 % agar in an independent holder on the micromanipulator so that after the micro-electrode had penetrated the bowel wall, the reference electrode could be advanced alongside it to make contact with the serosal surface. The potential difference across the bowel wall at the selected site could then be measured. The measurement was made using a 'Vibron' electrometer (Model 33B, Electronic Instruments Ltd.; input impedance 10^{13} Ω). The micro and reference electrodes were connected through Ag–AgCl junctions to the input of the electrometer, and before and after each penetration the balance of the electrodes was checked while their tips were immmersed in isotonic saline; pairs of electrodes with an asymmetry of greater than 0·5 mV were not used.

Analysis of faeces. The water content of the faeces was determined by drying in an oven at 98° C for 24 hr, preliminary experiments having shown that no further fall in weight occurred if drying was prolonged beyond this period. The dried material was transferred to silica tubes, extracted with concentrated nitric acid on a hot plate for 18 hr and then evaporated to dryness. The residue was dissolved in distilled water and the Na and K content

measured. In ten trial experiments, Na and K recovery estimated using this method was 98·2 ± 1·7 %. (The recovery of each element was similar and this value is based on the results for both. The mean is given here and as elsewhere when method accuracy is stated, it is given ± standard deviation.) To determine whether the Na and K measured in this way were freely diffusible, some fresh specimens from varous parts of the colon were extracted with distilled water or 0·3 M mannitol, shaking for 15 min and subsequently centrifuging. The supernatant was analysed for Na and K and the residue washed, dried and then extracted with concentrated nitric acid as described above and its Na and K content determined. Twelve specimens were examined and as there was no significant difference between the water and mannitol extraction all results were treated together. It was found that the percentage of Na extracted by water was 94·4 ± 2·6 and of K 72·5 ± 6·9. These results are similar to those of Wrong, Metcalfe-Gibson, Morrison, Ng & Howard (1965) on human stool using a dialysis method to determine diffusibility of faecal ions, and they indicate that practically all the faecal Na is in solution in the faecal water but that an appreciable proportion of K in rat faeces is in some 'bound' form, possibly within bacteria, vegetable material, etc.

Na and K were determined using an EEL flame photometer to an accuracy of ± 1·5 % (based on 10 replicates).

The results are given as means ± 1 standard error (S.E.) of the mean.

RESULTS

At the time of experiment, there was no significant difference ($P > 0·05$) between the control and Na-depleted rats in their plasma Na or K concentration (Table 1).

TABLE 1. Plasma Na and K in twelve normal and twelve Na-depleted rats. Results are given as mean ± 1 S.E.

	Plasma concentration (mM)	
	Na	K
Control	145 ± 1·1	4·8 ± 0·2
Na-depleted	143 ± 1·0	4·6 ± 0·2

Stool collection. When rats were transferred from rat cake diet (MRC 41 B) to the rice diet, the daily dry stool weight was approximately halved, probably a consequence of the reduced residue in the rice diet since the normal gain of body weight indicated that the rats ate adequately. The daily dry stool weight of the Na-depleted rats was less than that of the control rats, a result of their lower rice consumption (Table 2). The control rats lost in the stools a little more than 10 % of their Na intake and about 25 % of K intake, but when Na-depleted the Na loss was very much reduced although the K excretion changed little. K loss was increased, however, in proportion to dry stool weight. When rats took resin in the diet, the amount of Na and K in the stools rose considerably. In the control group, this was for the most part Na and these rats lost most of their Na intake in the stools. The Na-depleted rats lost much less Na in

the stools but still more than their intake and their stool K loss was greater than their K intake.

Analysis of fresh stools indicated a similar effect (Table 3). Water content could be determined on these stools; no difference was found between the controls and Na-depleted rats on rice alone. When rats were given rice with resin the water content was less ($P < 0.05$) and this was especially so with the Na-depleted group. In the control rats, the ratio Na/(Na+K) was considerably greater when the rats were fed a rice and

TABLE 2. Twenty-four hr stool excretion of Na and K of four normal and four Na-depleted rats when receiving rice alone and when eating rice+resin

	Diet	Mean stool dry weight (g/24 hr/rat)	Mean stool content (m-mole/24 hr/rat) Na	K	Na/(Na+K)
Control	Rice	1·65	0·16	0·28	0·37
Na-depleted	Rice	1·29	0·03	0·28	0·10
Control	Rice+resin	1·86	0·91	0·46	0·67
Na-depleted	Rice+resin	1·73	0·13	0·95	0·12

TABLE 3. Water, Na and K content of fresh stools of twelve normal and twelve Na-depleted rats. Results are given as mean ± 1 S.E.

	Diet	Stool water (% of wet weight)	Stool content (m-mole/kg water) Na	K	Na/(Na+K)
Control	Rice	70±3·3	48±3·4	64±10·2	0·42±0·05
Na-depleted	Rice	69±3·2	12±2·4	94±7·2	0·11±0·01
Control	Rice+resin	63±1·0	270±21	146±10·1	0·66±0·06
Na-depleted	Rice+resin	55±1·6	48±5·7	395±18	0·10±0·01

The normal and the Na-depleted rats were each divided into two groups of 6 rats. One group was fed on rice and the other was fed on rice to which resin had been added.

resin diet ($P < 0.001$) than when they were on rice alone. In the Na-depleted rats the opposite was true, the ratio being lower in the resin-treated rats ($P < 0.05$), but the difference was much less striking. The close agreement in the ratios between the fresh stools and the total stool collections in all the treatment groups indicated that fresh stools obtained at defaecation provoked by handling were reasonably representative of the natural stool.

Rat colon anatomy and contents. The terminal ileum entered the caecum close to the point of origin of the ascending colon (Fig. 1). The caecum was 2·5–3 cm in length with a capacity of 1·5–2 ml. when full. The ascending colon was 4–5 cm in length, its diameter varying according to its

contents but a glass tube of 0·7 cm o.d. could be passed through it without undue stretching. There was no defined transverse segment, the colon making an acute flexure and continuing as a descending segment of about 4 cm length. The diameter of the descending segment was a little less than the ascending, a 0·7 cm diameter tube being a tight fit, but a 0·6 cm diameter tube passing through easily. The final segment of about 3 cm length, and of similar diameter to the descending segment, passed from the brim of the pelvis to the anus. This segment was not easily accessible by an abdominal approach and was not studied in the present experiments.

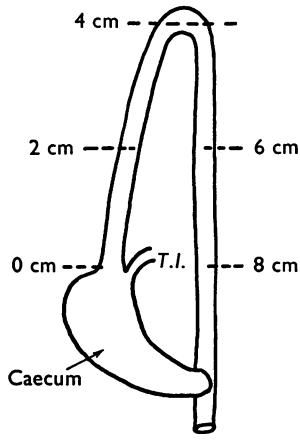

Fig. 1. Diagram to show the anatomy of the rat colon. The lengths refer to those of a 300–350 g rat. *T.I.*, terminal ileum.

The contents of the terminal ileum and caecum were semi-liquid. The terminal ileum was found to be empty in several rats but the caecum always contained some faeces. In the first 3 cm of the ascending colon the faeces were less liquid than in the terminal ileum but were still unformed; sometimes this part was empty. The formed stool bolus was not found before the final cm of the ascending colon.

Sometimes semiliquid faeces extended to the flexure but were not found beyond this point. Faecal boli were removed from various parts of the descending colon in two control and two Na-depleted rats and ten formed specimens obtained. They were ellipsoid in shape of mean length 1·4 cm (range 1·0–1·7 cm) and mean greatest diameter 0·6 cm (range 0·5–0·7 cm). The mean volume (measured by displacement in oil) was 0·42 ml. (range 0·30–0·51 ml.) and weight 0·51 g (range 0·33–0·59 g).

Transit time of faeces through the colon. The length of time the gut contents are in contact with the colonic mucosa must be important in determining the extent to which changes in faecal electrolyte composition can

occur. This is difficult to determine accurately and must in any case be variable. Some estimate was made, however, by adding carmine red to the rice in just sufficient amount to colour it pink and observing the time between its ingestion and its appearance in the caecum and the stools. Rats eat most of their daily food at night and it was found that kept under ordinary laboratory conditions they usually started to eat during late afternoon. Since in determining the transit time it was desirable that the rats should take their food in a natural manner rather than their being force-fed, the rats received carmine red rice in the evening. Four rats were studied and it was found that, although 10 hr after being given carmine red rice a few coloured stool boli had been passed, the majority were not dyed. The rats were then allowed no further access to the marked rice but nevertheless during the following 36 hr all their stools were coloured. A few days later the rats were again allowed to eat carmine red rice, it being provided in the late afternoon, and 6 hr later they were anaesthetized and the contents of the caecum and ascending colon inspected. It was found that the faeces in the caecum were coloured with carmine red in all the rats and in three there was also some dye in the first part of the ascending colon. These results suggest that transit through the upper intestine to the caecum in the rat is fast, the transit time being generally less than 6 hr, while transit through the colon is slow, the faeces spending about 6–30 hr passing through.

Composition of faeces from various parts of gut. In both the control and the Na-depleted rats, the percentage water in the faeces was greater in the caecal than in the terminal ileal specimen but thereafter fell progressively along the whole length of the colon (Table 4).

Na concentration in the terminal ileal samples was similar in the control and Na-depleted group but in the caecum there was a marked difference between them and this was maintained over the whole colon so that in the descending colon Na concentration in the controls was nearly 3 times as great as in the Na-depleted rats. K showed the reverse tendency, the concentrations being higher throughout the colon and caecum in the Na-depleted rats although the terminal ileal specimens were similar in the two groups.

From the data of Table 4 and the stool composition (Table 3) the net change in water, Na and K has been calculated for 1 g of caecal faeces as it passes through the colon (Table 5).

In this calculation it has been assumed that the material composing the dry weight does not change during passage through the colon, which, since the colon has no significant function in digestion and absorption other than in respect of water and electrolytes, is probably correct.

In the control rats most of the water and Na were removed in the first

TABLE 4. Water, Na and K content of material removed from various sites in the gut of six normal and six Na-depleted rats on a rice diet. Results are given as mean ± 1 S.E.

	Control				Na-depleted			
Site	Water (% of wet weight)	Na (m-mole/ kg water)	K (m-mole/ kg water)	No. of samples	Water (% of wet weight)	Na (m-mole/ kg water)	K (m-mole/ kg water)	No. of samples
Terminal ileum	81 ± 3·1	141 ± 8·5	24 ± 2·6	6	83 ± 1·2	137 ± 3·5	29 ± 2·2	4
Caecum	85 ± 2·1	111 ± 12·5	25 ± 2·1	6	87 ± 2·0	71 ± 5·6	53 ± 7·3	6
Cm along colon								
0–2	85 ± 2·2	78 ± 9·5	26 ± 3·6	4	84 ± 1·8	36 ± 2·9	66 ± 8·2	3
2–4	77 ± 3·0	64 ± 11·1	36 ± 6·1	4	82 ± 2·3	20 ± 4·7	64 ± 8·8	4
4–6	74 ± 3·8	52 ± 10·6	56 ± 5·9	4	72 ± 2·5	17 ± 1·8	82 ± 7·9	5
6–8	72 ± 4·1	48 ± 11·8	58 ± 4·8	3	69 ± 1·5	20 ± 2·1	84 ± 7·6	4

Usually various sites were empty so the number of specimens obtained from each site was frequently less than six.

4–6 cm (that is, in the ascending colon), less than 20 % of the water and 10 % of the Na being removed in the descending colon. This is consistent with the observations noted above that the faeces change from semiliquid to solid during their passage through the ascending colon. The same pattern was seen in the Na-depleted rats although the amount of water removed was more and of Na rather less than in the control rats. It must be noted, however, that the amount of Na in 1 g of faecal faeces presented to the colon was less in the Na-depleted rats and that the amount remaining in the faeces at 6 cm was very small. There was only a small change in Na and

TABLE 5. Water, Na and K content of a mass of material as it passed from the caecum to become stools in rats fed on a rice diet

	Faeces wet weight (mg)	Water (mg)	Na content (μmole)	K content (μmole)
		Control		
Caecum	1000	852	95	21
4–6 cm	576	428	22	24
Stool	488	340	17	22
		Na-depleted		
Caecum	1000	869	62	46
4–6 cm	467	336	6	27
Stool	420	289	3	27

The mass of material considered here started in the caecum at 1000 mg (wet wt.). The water, Na and K content were calculated from the data of Tables 3 and 4 and on the assumption that the dry weight remained constant, at 148 mg in the case of the control group and at 131 mg in the case of the Na depleted.

water content in the descending colon. Although the K concentration rose as the faeces passed from caecum to anus it is evident that this was due to water absorption since the amount of K changed little in the controls and in the Na-depleted actually fell. In both groups the amount of K changed little in the descending colon. When the concentration of the absorbed solution was calculated on the basis that only Na and K (with equivalent anion) were absorbed then in the ascending colon the estimated concentration was nearly isotonic to extracellular fluid but in the descending colon was substantially less.

Effect of resin. In the resin-treated control rats there was a high proportion of Na in the gut contents throughout the colon, although there was some reduction in the caecum and ascending colon (Fig. 2). Little change took place in the descending colon and the composition at its lower end was similar to that of the stools. The ratio Na/(Na + K) was considerably lower in the descending colon of the untreated rats when compared with the resin-treated controls ($P < 0{\cdot}005$), an observation similar to that noted when stool composition was determined (Tables 2, 3).

In the resin-treated Na-depleted rats (Fig. 3) Na predominated in the

terminal ileum but in the caecum and rest of the colon K predominated. The proportion of Na in the terminal ileum, caecum and ascending colon was significantly less ($P < 0{\cdot}05$) in the resin-treated than in the untreated but in the descending colon there was no difference.

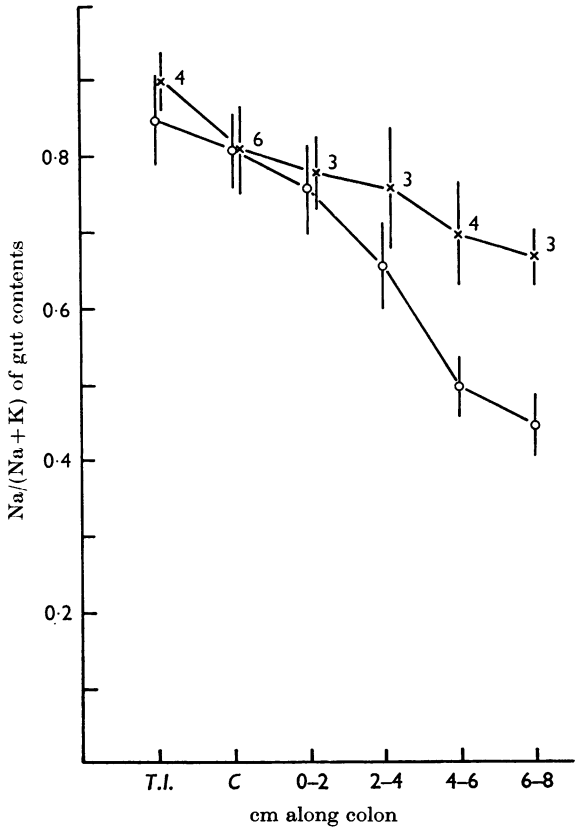

Fig. 2. The ratio Na/(Na+K) in gut contents removed from various parts of the gut of control rats. ×, Rats fed rice+resin; ○, rats on rice alone; *C*, caecum, *T.I.* terminal ileum. The numbers refer to the number of specimens for each point. They are not given for the rats fed on rice alone as these points were obtained from Table 4. Results are given as a mean ± 1 S.E.

The absorption of water, Na and K in the resin-fed rats during the passage of 1 g of caecal faeces through the colon was calculated (Table 6). The method of calculation was the same as that employed in the construction of Table 5, it being assumed that the dry weight was unaltered during passage through the colon. There was considerable water absorption in both groups of animals although greater in the Na-depleted. Most of this absorption occurred in the ascending colon, a finding similar to that

with rats fed on rice alone. The amount of Na absorbed by the colon was greater in the control rats, but as there was much less Na present initially in the caecum of the Na-depleted group the amount of Na in the stools was very low in this group. In the controls most of the Na was absorbed in the ascending colon but in the Na-depleted the ascending and descending absorbed about equal amounts. A striking difference in behaviour of the

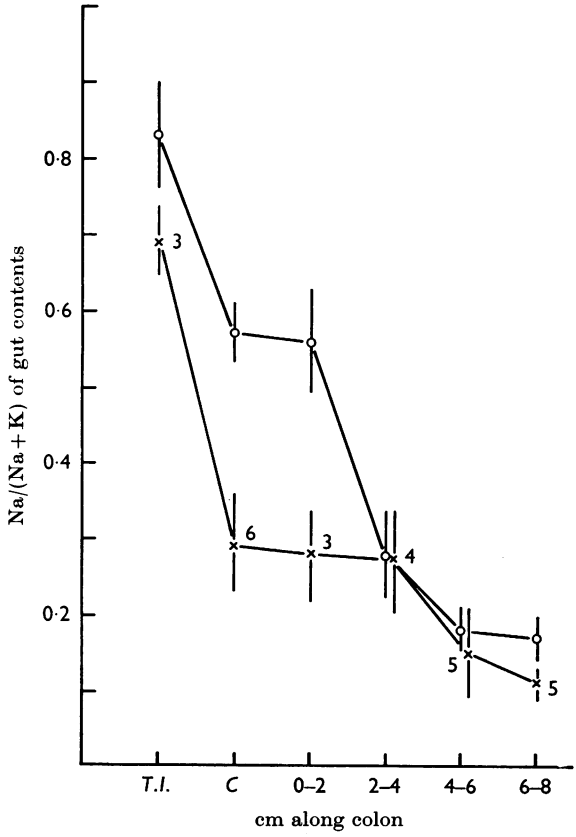

Fig. 3. The ratio Na/(Na + K) in gut contents removed from various parts of the gut of Na-depleted rats. The symbols are as Fig. 2.

two groups was in regard to K absorption. In the controls the amount of K increased continuously from caecum to stools, indicating secretion in both colonic segments, but in the Na-depleted rats the K content fell in the ascending colon and changed little in the descending.

Electrical potential measurements. All the potentials observed in these experiments had the same polarity, the serosal surface of the bowel being positive with respect to the luminal. The colon wall has several layers—

peritoneum, muscle, connective tissue and mucosa—and it was necessary to establish the tissue of origin of the observed p.d. In several experiments, an additional reference electrode was placed in the jugular vein (I.V. electrode). The p.d.s between the I.V. and the mucosal-side and serosal-side

TABLE 6. Water, Na and K content of a mass of material as it passed from the caecum to become stools in rats fed with rice diet, containing resin

	Faeces wet (mg)	Water (mg)	Na content (μmole)	K content (μmole)	n
		Control			
Caecum	1000	784 (78 ± 2·8)	192 (245 ± 14)	31 (40 ± 6·6)	6
6 cm	619	403 (65 ± 2·3)	110 (275 ± 22)	45 (111 ± 14)	3
Stool	588	372	100	54	6
		Na-depleted			
Caecum	1000	775 (77 ± 3·1)	54 (70 ± 18)	131 (170 ± 26)	6
6 cm	582	357 (61 ± 2·4)	36 (108 ± 11)	102 (285 ± 29)	4
Stool	501	276	13	109	6

The water, Na and K content were calculated in the same way as in Table 5. The values in brackets are the actual values found from analysis of the specimens obtained from the caecum and from the 6 cm region of the colon. They are given as a mean ± 1 S.E., n being the number of specimens. The water is expressed as % wet weight and Na and K as m-mole/kg water.

TABLE 7. Potential difference (mV) between lumen and serosal surface of caecum and colon in normal and Na-depleted rats on a rice diet with or without the addition of resin

			Potential difference (mV)			
			Ascending		Descending	
	Diet	Caecum	0–2	2–4	4–6	6–8
			cm along colon			
Control	Rice	14·2	10·5	9·2	10·3	13·6
		6–23	7–15	7–14	8–15	6–18
	Rice + resin	12·8	11·8	11·6	15·4	18·9
		7–18	9–15	9–14	12–18	13–25
Na-depleted	Rice	40	17·6	14·5	25·4	38
		34–44	10–25	10–21	15–36	28–52
	Rice + resin	37	18·3	16·0	64	50
		32–44	16–23	12–19	44–115	39–72

Mean values are given with the range of observations. Serosal surface was always + ve with respect to lumen.

electrodes were measured. No significant p.d. existed between the I.V. and the serosal-side electrode, but a p.d. of the same polarity and size as the transmucosal p.d. was obtained between the I.V. and the mucosal-side electrode, suggesting that the observed p.d. originated in the mucosa. More direct evidence was obtained by applying the reference electrode directly

to the mucosa. On several occasions, both in control and Na-depleted rats, the muscular coat was incised longitudinally using a fairly blunt scalpel and the muscle gently separated from the underlying mucosa over a small area. It was then possible to place the reference electrode on the mucosa. In these experiments it was found that the full transmucosal p.d. was measured so that the peritoneum and muscular layer could not have been contributing to the observed p.d. It was concluded therefore that the observed p.d. originated in the mucosa.

Caecum. The transmucosal p.d. was measured at five points separated from each other by about 0·5 cm along the caecum of each rat. For any given animal, control or Na-depleted, the readings varied little between different points but there was considerable variation between animals as indicated by the range of the measurements (Table 7). The p.d. values of the Na-depleted group were substantially higher than in the controls, and whether the animals' diet contained resin or not did not appear to make any significant difference to the p.d. in either group.

Ascending colon. Potential differences in the Na-depleted rats were in general greater than in the controls, although occasionally in the latter, potentials as high as those found in the Na-depleted were observed. There was little variation over most of the length of the ascending colon in both groups although it was often observed that the first centimetre tended to have a higher potential than the remainder; this difference was small in the control rats but was exaggerated by Na depletion.

Descending colon. In the control group the mean p.d. in the descending colon was a little higher than in the ascending colon, but there was little variation along the length of the bowel in either part of the gut. When the rats were on resin the p.d.s were somewhat greater, although considerable variation among the animals made it uncertain whether this was a real effect. The most striking change was produced by Na depletion. In the first place, the potentials were considerably higher; secondly, they tended to increase from the proximal to distal end of the bowel; and thirdly, they were substantially greater in rats receiving resin than those on rice alone. The highest p.d.s observed in these experiments, greater than 100 mV, were measured in the descending colon of Na-depleted rats taking resin and were twice as great as the maximal values found in the rats on rice alone.

The general tendency was for the p.d. in Na-depleted rats to increase from proximal to distal end but this was not always true. Figure 4 shows the measurements in one Na-depleted rat on resin in which the highest potentials were seen at about 6–7 cm. The p.d. was lower at points along the bowel which were empty and the effect was most obvious in the Na-depleted rats. This is illustrated in Fig. 4, where the empty segment had a

p.d. of only 21 mV while the adjacent filled regions gave 63 and 112 mV. In part, at least, this may have been due to a low concentration of Na in the lumen of the empty segments since injection of 150 mM-NaCl into the bowel consistently produced a rise of p.d. Injecting this solution into one of the empty segments in the case of the experiment of Fig. 4, for example, elevated the p.d. from 21 to 46 mV.

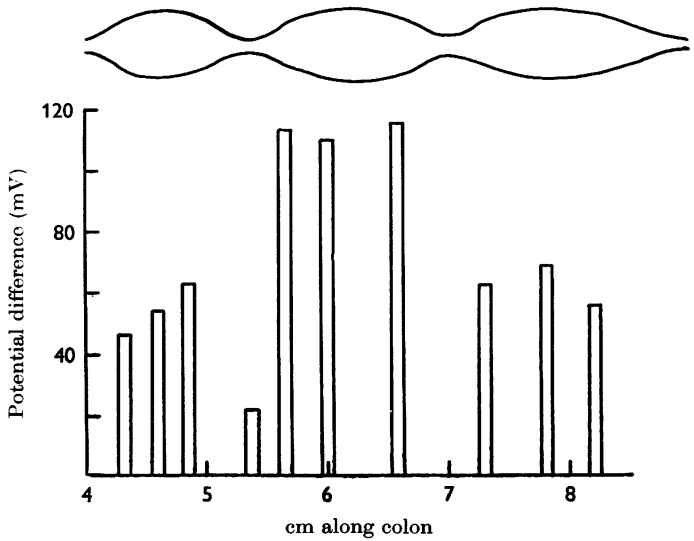

Fig. 4. An example of p.d. measurements made in the descending colon of a Na-depleted rat fed with rice+resin. The rat illustrated was somewhat unusual in having the highest p.d. values at about the middle of the descending colon. The figure shows diagrammatically the appearance of the faecal boli in the gut with short empty segments between, the latter having relatively low potentials.

DISCUSSION

Sodium and water. During the passage through the rat colon, the faecal material is converted from a medium containing a large proportion of Na and little K to one containing less Na than K. The change is similar to that observed in dogs (Field, Dailey, Boyd & Swell, 1954) and in man (Sammons, 1961; Kramer & Ingelfinger, 1961) when the composition of ileostomy fluid was compared with that of stools. To effect these changes Na and water must be absorbed by the colonic mucosa, most of the absorption taking place in the ascending colon. The concentration of Na was however much lower in the descending colon and this may have been responsible for its absorptive capacity being apparently less.

In contrast to the findings of Field *et al.* (1954) in dogs, no significant difference in composition of the ileal fluid is induced by Na depletion in

rats, so that the striking difference in stool composition produced by Na depletion is due to a change in absorption by the colonic mucosa. The electrochemical gradient across the colon wall is considerably increased by Na depletion since both the luminal Na concentration is reduced and the electrical p.d. elevated. Despite this, the absorption of Na in both segments of colon is only a little less in Na-depleted rats. It appears likely therefore that active Na transport is stimulated by Na depletion.

TABLE 8. Comparison of the K concentration (mM) observed in the water of the gut contents with that calculated from the Nernst equation using the plasma K concentration and the observed p.d.

	Control		Na-depleted	
Site	Calculated	Observed	Calculated	Observed
Caecum	8·1	18·2	20·7	38·5
Cm along colon				
0–2	7·3	18·9	9·1	48·3
2–4	6·7	26·5	8·1	47·2
4–6	7·0	40·5	11·8	59·8
6–8	8·1	42·5	19·1	61·4

To obtain the observed K concentration, the observed K values of Table 4 were multiplied by 0·73. This allowed for the fact that not all the K was in free solution.

Potassium. The interpretation of the high K content of the faeces is more difficult since the electrical p.d. favours the development of a K concentration within the gut lumen greater than that of the plasma. If, however, the K concentration in the faecal water develops simply as a result of the plasma K and the electrical p.d., the colonic mucosa behaving essentially as an inert membrane with regard to the ion, then the equilibrium value can be calculated from the Nernst equation:

$$[K]_l = [K]_p \exp. (\Delta VF/RT),$$

where $[K]_l$ is the luminal and $[K]_p$ the plasma K concentration, ΔV is the electrical p.d. across the colonic wall and the other symbols have their usual significance. (The ionic strengths of faecal solution and plasma are similar and so the activity coefficients have been omitted from the equation.) As is apparent from Table 8, the observed value of $[K]_l$ was always considerably greater than was anticipated from calculation. The discrepancy could not be due to the solvent drag of water absorption as this effect would tend to reduce $[K]_l$ to less than the calculated value. The major difficulty is to know whether the observed luminal K concentration represented an equilibrium value or was produced by a rate of water absorption much faster than that of K. Water absorption was occurring throughout the colon so that a rise of K concentration could have been due simply to reduction in volume of faecal water and such an explanation was tenable in the rats fed on rice only since in them there was little change in

luminal K content. A suggestion that the concentrating effect of water removal is not the complete explanation comes from consideration of results of experiments on resin-fed normal rats, for in these animals K was added to the gut contents over the whole length of colon while the ratio K/(Na+K) increased from 0·16 in the caecum to 0·35 at the end of the descending colon. When a resin is immersed in an electrolyte solution, ions of the fluid phase rapidly equilibrate with it (Kitchener, 1961) so that, using the resin's selectivity coefficient, the K/(Na+K) ratio of the faecal fluid can be estimated at 0·13 in the caecum and 0·28 in the descending colon. Taking the total (Na+K) concentration in the fluid phase to be the same as in the control rats not on resin, then these proportions correspond to a K concentration of 17 mM in the caecum and 28 mM in the descending colon (allowance has been made in this calculation for the fact that only 73 % of the total K is in free solution in the faeces of normal rats not eating resin). Thus K secretion was occurring although the luminal K concentration was greater than the calculated equilibrium value. This suggests that water absorption is not the complete explanation of the high K concentration observed in the faeces but that there is active transport of K into the colonic lumen.

Potential difference. In several electrically polarized epithelial membranes the p.d. has been related to active Na transport (e.g. Ussing & Zerahn, 1951; Windhager & Giebisch, 1961) and such a mechanism has been suggested to account for the colonic p.d. (Cooperstein & Brockman, 1958; Curran & Schwartz, 1960). A rise of p.d. could result from an increase of Na absorption with tissue passive permeability remaining constant. The p.d. rise induced by Na depletion was not, however, associated with increased Na absorption although the work of Na absorption was increased due to the elevated electrochemical gradient. It is unlikely that K movements were responsible for the p.d. change since while the high luminal K concentration of faecal water could produce a diffusion potential of correct polarity, this was evidently not important because the high p.d. of Na depletion is found even when luminal K concentration is very low (Edmonds, 1967*a*). It seems likely that the elevation of p.d. is related to stimulation of active Na transport but the mechanism by which it is brought about is at present obscure.

Changes in faecal composition and transit time. Using the Na and K net flux values obtained from perfusion experiments (Edmonds, 1967*a*, *b*) it is possible to estimate the time necessary for the observed changes in faecal Na and K composition to take place. In this estimate, the data of Table 5 were used. The faecal boli removed from the lower descending colon averaged about 500 mg wet wt., and in Table 5 the faecal material which started in the caecum at a wet wt. of 1000 mg was reduced by water

absorption to a wet wt. of about 500 mg in the descending colon. Table 5, then, relates the history of an average bolus as it passes through the colon. Judged from its dimensions, a bolus of this size makes contact over about 1·5 cm of length of colon mucosa. During passage of the bolus through the whole colon, the normal rats absorbed 78 μmole Na at a luminal concentration from 111 mM in the caecum to 48 mM at the end of the descending colon. Over this concentration range the Na absorption rate has a mean value of about 50 n-mole/min/cm (Edmonds, 1967*a*) and hence the time required to absorb the Na from a bolus would be $78/(0{\cdot}05 \times 1{\cdot}5)$ min or about 18 hr, which is in fair agreement with the observed transit time through the colon. When faeces enter the colon the K concentration in the faecal water is only a little less than the luminal K concentration associated with zero net K flux (Edmonds, 1967*a*). Consequently, K secretion will be at a low rate. As water is absorbed the K concentration rises so that K now tends to be absorbed. Hence the over-all effect anticipated would be one of little change in luminal K content in normal rats and this was in fact observed.

In Na-depleted rats the luminal Na concentration is much lower, from 71 mM in the caecum to 20 mM at the end of the descending colon. Over this range the mean Na absorption rate of the normal colon is practically zero (Edmonds, 1967*a*) so that an increase of Na-absorbing capacity of the colonic mucosa must be postulated to explain the observation that the amount of Na removed from the faeces is almost the same in these rats as it is in the controls.

The inclusion of resin in the diet means that a fairly large amount of unabsorbable anion passes through the colon requiring cations as counter-ions. The resin rapidly equilibrates with a solution into which it is placed so that in the gut the counter-ions of the resin are in equilibrium with the cations of the faecal water phase. In the normal rats, however, the proportion of Na in the gut contents was found to be much greater in the resin-treated than in the untreated rats (Fig. 2). The stool Na content of normal rats on resin was also greater than expected from ratio Na/(Na + K) in stools of rats not taking resin. In the Na-depleted rats, on the other hand, the stool Na was as expected. Table 6 shows that in normal rats about 50 μmole Na/bolus more must be absorbed by the colon and the same amount of K secreted for the stool Na/(Na + K) ratio to be the same in the resin treated as in the rats on rice alone. The relatively high Na content of the stools of the resin-treated rats may therefore have resulted from inadequacy either of Na absorption or K secretion. Inadequate Na absorption seems unlikely since the Na concentration in the faecal water of the resin-fed rats was high. That inadequate K secretion was responsible is clear from the following considerations. The normal secretion rate at the

K concentrations present in the colon would be not greater than about 10 n-mole/min/cm (Edmonds, 1967*b*). For a 1·5 cm long bolus this would mean that about 55 hr are required for 50 μmole of K to be secreted. The observed K secretion was less than half this amount, which agrees well with the transit time of faeces through rat colon. Thus in the time available the amount of K secreted was the limiting factor and Na remained in the lumen as the obligatory counter-ion.

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