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SODIUM TRANSPORT AND SHORT-CIRCUIT CURRENT IN RAT COLON *IN VIVO* AND THE EFFECT OF ALDOSTERONE

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SUMMARY

1. A method for measurement of short-circuit current and for applying a voltage clamp to segments of rat colon *in vivo* is described.

2. The mucosa behaved as an ohmic resistor of average resistance 154 Ω/cm^2 although brief transient effects were frequently observed. Tissue resistance was independent of considerable changes in ionic strength and composition of the luminal solution.

3. The short-circuit current averaged 120 $\mu A/cm^2$ in normal rats. Aldosterone intravenously raised the p.d., short-circuit current rising proportionately and tissue resistance being unchanged. The effects of various modifications of the intraluminal solution in respect to composition, hydrostatic pressure and pH were examined. An increase in the osmolality of the luminal solution sufficient to abolish water absorption did not affect p.d. or short-circuit current.

4. The short-circuit current measured with 150 mM-NaCl in the lumen was almost completely accounted for by active Na absorption both in normal and aldosterone-treated rats. The changes in Na efflux rate produced by voltage clamping suggested that only part of Na efflux was due to simple diffusion. With lower [NaCl] in the lumen, the short-circuit current exceeded that atributable to active Na absorption, the discrepancy increasing with reduction of [NaCl].

5. The luminal [Na] at which Na efflux and influx rates were equal was reduced by aldosterone, an effect which is probably responsible for the low stool [Na] of aldosterone treated animals. The significance of this finding in terms of the mode of action of aldosterone is discussed.

INTRODUCTION

The use of the short-circuit current method introduced by Ussing & Zerahn (1951) for investigations on frog skin has been of considerable importance in the development of modern views on the mechanism of ionic transport across epithelia. The technique has been applied predominantly to *in vitro* studies. In the case of amphibian epithelia, since the tissues survive for many hours after isolation, it seems probable that the *in vitro* resembles the *in vivo* condition. In the case of mammalian epithelia, however, this is less certain. Mammalian intestinal epithelium, for example, tends to deteriorate quite rapidly after removal from the animal, the p.d. beginning to fall shortly after isolation and usually having almost disappeared in 1–2 hr. Further, a number of observations have suggested that considerable differences may exist between *in vivo* and *in vitro* behaviour of the same tissue. The ileum of intact animals, for example, appears to absorb Cl against the electrochemical gradient yet the isolated epithelium does not have this property (Dennis & Visscher, 1940; Curran & Solomon, 1957; Clarkson & Toole, 1964). In investigating the action of aldosterone on Na transport by mammalian colon the disadvantage of using isolated epithelium was particularly apparent since no response to the hormone was found *in vitro* although *in vivo* considerable changes were observed (Edmonds & Marriott, 1967, 1968*a*). In the present study we have therefore used a method which allows the short-circuit current technique to be applied to the living animal so that the effects of aldosterone could be investigated *in vivo* and relationship between Na transport and the short-circuit current determined.

METHODS

Male albino rats weighing 300–350 g were used. They received a standard rice diet which was prepared as 1 g tablets of composition rice 49 %, glucose 49 %, casein 1 %, KCl 0·2 %. The composition of the diet was slightly modified from that used in previous experiments (Edmonds, 1967*a*) but its preparation as tablets had considerable production, storage and handling advantages.

The methods of gut cannulation and fluid circulation were based on those used previously (Edmonds, 1967*b*), the distal cannula communicating with a small reservoir and the perfusion fluid recirculated by a peristaltic pump, through a water-bath maintained at 38° C before re-entering the bowel through the proximal cannula. In the present experiments, short segments of descending colon about 2 cm long were used.

Measurement of short-circuit current. The cannulae were modified to include electrodes for measuring p.d. and passing current during the perfusion (Fig. 1). The distal cannula was a glass tube (o.d. 5 mm, length 6 cm), which was inserted into the colon through the anus. The external end connected with a glass Y-tube (o.d. 5 mm) one arm of which fed fluid into the circulation reservoir. The other arm connected with

the current-passing electrode which consisted of a Perspex tube (o.d. 5 mm, length 2 cm) filled with 150 mM-NaCl–4 % agar jelly containing a silver plate coated with AgCl and connecting with a battery and potentiometer. A microammeter was included in the circuit to measure the current passed through the tissue. The circuit was completed by clipping the other lead to a silver plate coated with AgCl, the latter being wrapped in gauze soaked in 150 mM-NaCl and stitched to the subcutaneous tissue through a small skin incision in the neck. The proximal cannula was a polyethylene tube (o.d. 3 mm) mounted inside a short length of glass tubing (o.d. 5 mm) so that at one end the two were flush and at the other the polyethylene

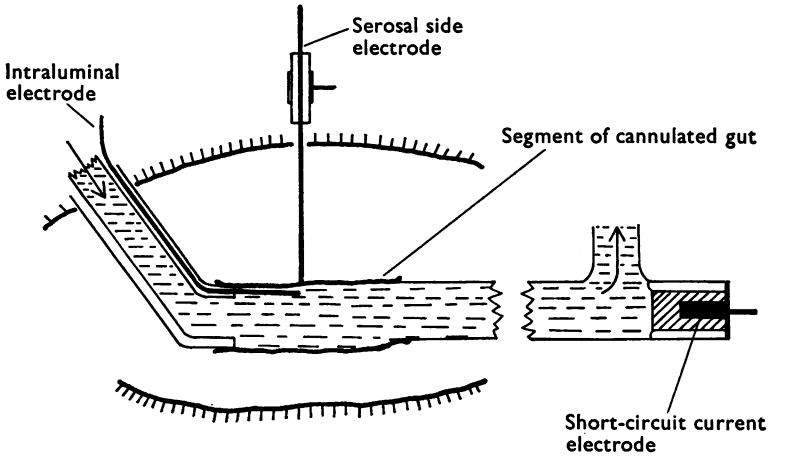

Fig. 1. Diagrammatic representation of method of measuring short-circuit current of rat colon *in vivo*.

tube was free to connect with the fluid circulation system. Another polyethylene tube (o.d. 1 mm) filled, in the majority of experiments, with 150 mM-NaCl–4 % agar, was inserted between the polyethylene and glass tubes so that the tip protruded by about 8 mm. This formed the luminal side electrode and it was fixed in position between glass and polyethylene tubing by Bostik cement which also served to seal the connexion, ensuring that the perfusion fluid passed only through the central polyethylene tube. The free end of the p.d. measuring electrode was connected through a calomel bridge to a Vibron electrometer (model 33B E.I.L.). The reference electrode was a polythylene tube (o.d. 1 mm) filled with 150 mM-NaCl–4 % agar which made contact with the peritoneal surface of the colon segment. It was held in position by a micromanipulator and so could be placed exactly opposite luminal electrodes. It was connected through a calomel electrode to the low input of the electrometer. The asymmetry between the intraluminal and reference electrodes was always checked before experiments by placing their tips together in saline and did not exceed 1 mV. In some experiments two electrodes were placed in the gut lumen enabling the transmucosal p.d. to be monitored at two points along the gut at about 1 cm apart. It was clear from these trials that there was little variation in p.d. along the gut segment during the experiments, presumably because the cannulated segment was only about 2 cm in length and so only one luminal electrode was used in most studies. It was important to ensure that the tips of the luminal and serosal side electrodes were exactly opposite each other, the luminal side electrode being painted black to facili-

tate accuracy of positioning. One to 2 ml. NaCl, 150 mM, was placed in the abdominal cavity which together with small intestinal segments lying adjacent to the colonic segment ensured that there was an even density of current flow through the intestinal wall. Preliminary experiments using an electrode which was freely movable within the lumen confirmed that under these conditions when current was passed the transmucosal p.d. could be reduced to zero at all points of the colonic segment. During passage of current, a fall of p.d. was present along the length of the animal. For example, when passing 200 μA there was a voltage drop of nearly 950 mV between the two current passing electrodes. There was a fall of 50–60 mV between the neck electrode and the proximal cannula, 5–10 mV along the cannulated segment and about 850 mV along the length of the distal glass cannula. Some error in estimating the short-circuit current occurs as a result of the resistance of the saline present between the tips of the recording electrodes. However, preliminary experiments showed that since the electrical resistance of colon was relatively high, the error introduced was very small and so no correction was made in the reported results.

Sodium flux measurements. The principle of the present method was first to measure the Na influx rate (flux from lumen to plasma, J_i) by placing a ^{22}Na containing solution in the lumen, and subsequently to measure the Na efflux rate (flux from plasma to lumen J_o) after ^{22}Na had been injected into the animal. Duplicate determinations of both influx and efflux rates were made during periods of open circuit, that is when no external current was applied and during periods of short-circuiting when a current was passed through the tissue sufficient to reduce the transmucosal p.d. to zero. Measurements of J_i and J_o were done both while solution was being circulated through the gut lumen and simply with the solution placed in the lumen without circulation. No significant differences were found between the circulating and non-circulating methods and so all the results have been grouped together. In circulation experiments, J_o was determined by circulating 4–5 ml. fluid at a rate of about 2·5 ml./min for 20 min. The solution was then removed completely by suction and the lumen rinsed through with 20 ml. isotope-free NaCl, 150 mM; the amount of ^{22}Na absorbed was then measured by a whole body counter (Barnaby & Edmonds, 1969). In all the experiments, the amount of ^{22}Na used in the lumen was about 200 nc/ml. J_o was determined by giving 3 μc ^{22}Na in 0·3 ml. NaCl, 150 mM I.V., and 1 hr later circulating known volumes of fluid for 20 min and measuring the amount of ^{22}Na appearing in the luminal fluid by taking an aliquot and measuring its ^{22}Na content. A single blood sample only was taken at the end of the experiment since preliminary studies indicated that there was little change in plasma Na specific activity between 1 and 3 hr after injection of ^{22}Na. In the experiments in which there was no circulation, after preliminary rinsing with the test solution, some solution was introduced into the lumen so as to fill the gut segment, care being taken to ensure that no air bubbles were present. After 20 min the solution was removed. When J_i was being measured, the lumen was rinsed with 20 ml. NaCl, 150 mM, before whole body counting. When J_o was measured, the solution removed was collected by gentle suction into a small test-tube and a further 5 ml. solution was passed through the segment and collected to ensure that the ^{22}Na which had entered the lumen was removed. The present technique had the disadvantage that the unidirectional fluxes were not measured simultaneously. It had, however, the considerable advantage that the Na influx rate could be measured by whole body counting, a method which is simple and which allows accurate measurement of uptake of radionuclide. This was particularly important since the uptake was small in the present experiments as short pieces of gut were used with only brief periods of exposure to test solutions. The technique would, however, be unsatisfactory if

during the 4–4½ hr required to complete the measurements considerable variations in J_o occurred. In preliminary assessments involving estimation of J_o repeatedly over several hours, although some variation in a series of values of J_o on any animal was apparent, the variation was relatively small and random, the range not exceeding ± 18 % of the mean value. Thus values for J_o obtained in two consecutive measurements could differ by as much as if they were separated by several hours. It therefore appeared that the average of two 20 min estimations of J_o determined later gave a fair reflexion of the average flux rate obtaining over the whole period of the experiment.

Aldosterone administration. Aldosterone (Aldocorten, Ciba) was given by I.V. infusion through a polyethylene cannula (o.d. 0·5 mm) which was implanted into the external jugular vein between 2 and 5 days before experiments, the free end of the cannula being exteriorized through the back of the neck. When aldosterone treatment is referred to in this paper, it means a 5-hr I.V. infusion of aldosterone, 10 μg/hr.

Luminal solutions. Solutions placed in the lumen of various [Na] were prepared from stock solutions made with A.R. reagents, all solutions being rendered isoosmotic with NaCl 150 mM, by addition of mannitol. The solutions were not buffered as variation of pH over the range 5–8·5 was found to have no effect on p.d. short-circuit current or tissue resistance.

Radionuclide measurements. Liquid samples were pipetted in 0·2 or 0·5 ml. volumes on to Al planchets, dried in an oven (98° C) and the radioactivity measured using an automatic End Window G.M. counter (Panax Instruments Ltd.). Tissue samples were placed flat on Al planchets and similarly dried and their radioactivity measured. To determine absorbed ^{22}Na a whole body counter was employed, the animals being placed in the counter for 2–3 min. The counter and its use for the measurement of intestinal absorption together with various calibration procedures have been previously described (Barnaby & Edmonds, 1969).

Chemical methods. Na and K content of samples of plasma, tissue and test solutions were determined using a Gallenkamp flame photometer, osmolality by a Fiske osmometer and pH by glass electrode and an E.I.L. pH meter. Phenol red was contained in some solutions as an indicator of water movement; its concentration was measured as previously described (Edmonds, 1967*b*).

Calculations. Flux rates are expressed per square centimetre, the area of the segment being obtained by measuring its length on a standard glass tube (o.d. 7 mm) with the tissue fully expanded but not stretched and multiplying the length by 2·1. Results are given as mean ± 1 S.E. of the mean.

RESULTS

Short-circuit current, p.d. and electrical resistance

The blood supply of the descending colon is derived from branches of both the superior and inferior mesenteric arteries. The segment of intestine used in the present experiments was the distal part of the descending colon. Ligature of the branches from the superior mesenteric artery alone caused no significant change in p.d. or in short-circuit current. When the inferior mesenteric artery was also ligatured, thus stopping all blood circulation through the descending colon, p.d. and short circuit current began to fall after several minutes. The fact that there was always a delay of at least 2–3 min before any change was observed, indicated that the presence

of circulation in the tissue itself did not influence the measurements. P.d. and short-circuit current fell almost in parallel, probably as a consequence of oxygen lack since a similar effect on p.d. was observed when animals were given nitrogen to breathe (Edmonds & Marriott, 1968*a*).

Electrical resistance. The tissue resistance was measured by passing a known direct current through the tissue and measuring the p.d. change across it. The tissue resistance was measured periodically during the experiments which, when flux measurements were made, lasted 4–4½ hr.

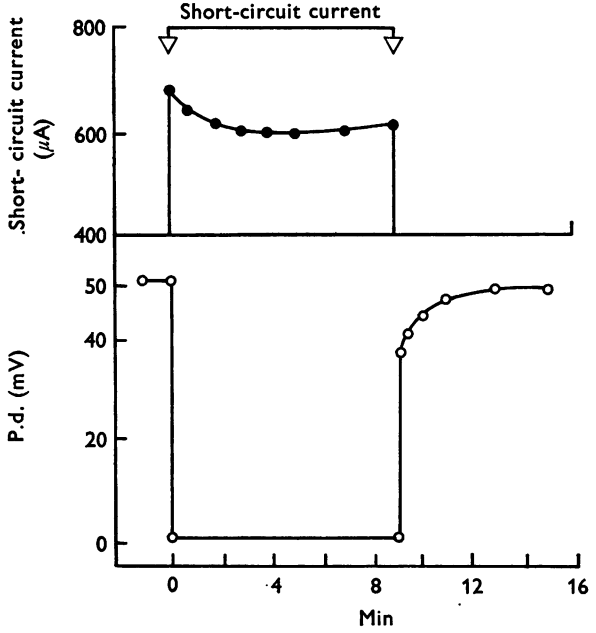

Fig. 2. Effect of applying short-circuit current illustrating transient changes particularly evident when the p.d. was high. The results are taken from an experiment on an aldosterone-treated rat.

No evidence of any significant changes in resistance was found despite this fairly prolonged exposure to a saline solution in the lumen. The p.d. of the tissue altered little during the course of the experiment and the short-circuit current also underwent little change although experiments lasted several hours. When current was first passed, it was often observed that the initial value of the short-circuit current was not maintained but tended to fall rapidly to reach a steady lower level (Fig. 2). Also when the current was switched off, the transmucosal p.d. rose rapidly at first to a value less than before current application although it then usually returned slowly to the former level over 2–3 min (Fig. 2). The transient effects were nearly

always present to some degree but were most obvious when transmucosal p.d. was relatively great (> 30 mV). These transients were comparable to those noted by Cooperstein & Hogben (1959) using an *in vitro* preparation of frog colon. Their presence suggested the existence of capacitance in the mucosa but they were not investigated in detail in the present work. Attention was given primarily to the steady-state condition, and as far as this was concerned the findings were similar to those obtained from colonic mucosa *in vitro*, the p.d. change being always proportional to current

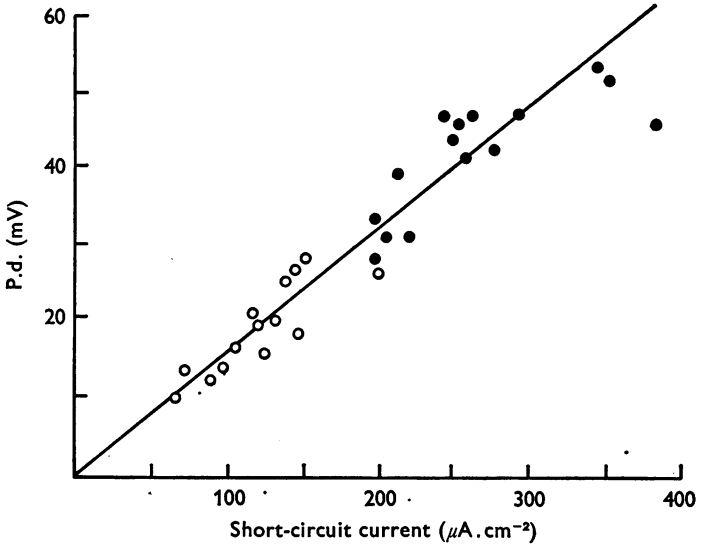

Fig. 3. Relationship between short-circuit current and p.d. of descending colon of fourteen normal and fifteen aldosterone-treated rats. The regression line was calculated by the least-squares method. ○, normal; ●, aldosterone-treated.

change both in normal and aldosterone-treated rats and irrespective of direction of current flow. The mucosa therefore behaved essentially as an ohmic resistance. The tissue resistance was found to be similar in normal and aldosterone-treated rats although the p.d. and short-circuit current were considerably greater after aldosterone treatment (Fig. 3). Tissue resistance was 154 Ω/cm^2, the area being that of the serosal side and measured in the standard way described. The colon mucosal epithelial cells are covered with microvilli on the luminal surface so that the actual area of cell membrane in contact with the luminal solution must be very much greater than we measured. The value for tissue resistance obtained in these *in vivo* experiments was rather higher than previously observed with an *in vitro* preparation, when at 32° C the mean value was 108 Ω/cm^2.

Probably as with small intestine (Barry, Smyth & Wright, 1965) the electrical resistance of the tissue drops appreciably when it is prepared *in vitro*. Although it is difficult to make precise comparisons since the techniques differ, the present values of electrical resistance for rat colon are substantially higher than those given for small intestine (Barry *et al.* 1965; Asano, 1964) which were of the order of 30–60 Ω/cm^2.

In all these measurements the serosal side electrode was separated from the epithelial cells by smooth muscle, connective tissue and peritoneum. If these tissues outside the epithelium contributed significantly to the over-all resistance of the colon wall, an appreciable error could be introduced into the measurements of resistance and short-circuit current. Several experiments were carried out to examine such a possibility. Using a sharp scalpel blade, the peritoneum and smooth muscle layer were incised and stripped away from the underlying mucosa in the living animal. What little bleeding occurred usually ceased within a few minutes. The p.d. and short-circuit current were virtually unaffected by the procedure and it seemed reasonable to conclude that practically all the resistance to current flow was in the mucosal cell layer.

Effect of various changes in the luminal solution

Hydrostatic pressure. The effect of pressure within the segment of intestine was examined by connecting the distal cannula to a manometer. When the intraluminal pressure was very low, about 2–3 cm of saline or less, the short-circuit current was less than at higher pressures. However, with such low pressures the intestine was lax. With a pressure of 5 cm saline, the gut appeared full and the short-circuit current reached a value which did not change even when the intraluminal pressure was raised high as 30 cm saline. P.d. and tissue resistance were also unchanged. Thus it seemed that providing the gut was fully distended, changing intraluminal hydrostatic pressure over a range up to 30 cm had no significant effect. In the case of frog skin, it has been found that even a small hydrostatic pressure applied to the outer surface of the skin and maintained for 30 min or more could produce a rise of short-circuit current persisting even when pressure was discontinued (Nutbourne, 1968). We could demonstrate no such effect in the colon. With hydrostatic pressure maintained at 5 cm or at 15 cm for up to 1 hr no significant change in p.d. or short-circuit current was found. When the pressure was greater (about 40 cm) the p.d. fell gradually and by about 30 min it had sometimes dropped to nearly half of its initial value. The short-circuit current changed little, however, and the fall of p.d. resulted from reduced tissue resistance (Fig. 4). These changes were present for at least an hour after pressure was removed and probably resulted from damage to the mucosa itself.

Composition of the luminal solution. The effect of several variations in the composition of the solution within the lumen was examined in a number of experiments. The substitution of Krebs–Ringer solution with a composition more closely approximating to that of the extracellular fluid which bathed the serosal side of the mucosal cells, did not significantly influence the p.d. or short-circuit current. Hence in the majority of experiments, unbuffered NaCl 150 mM was employed despite the fact that its use meant that there

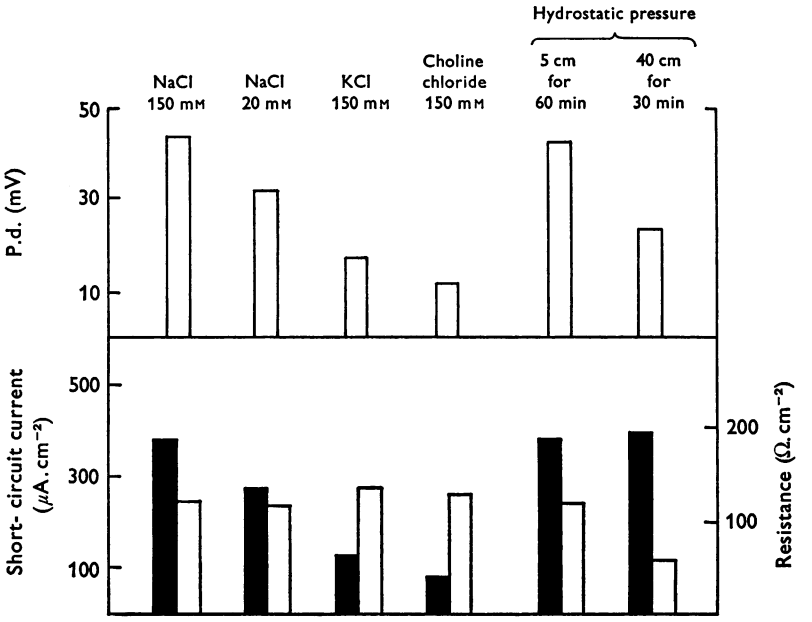

Fig. 4. Effect of altering ionic composition and hydrostatic pressure of the luminal solution on the p.d., mucosal electrical resistance and short-circuit current. The results are shown for an aldosterone-treated rat, mean values being obtained during two or three exposures to each solution. Five minutes were allowed for p.d. and short-circuit current to become constant after introducing fresh solution. In the experiments on pressure effects, the lumen contained NaCl 150 mM, and measurements were made after the pressure had been reduced to a level of about 3 cm water. In the lower part of the Figure, the open columns indicate tissue resistance and the filled columns, short-circuit current.

must have been some small ionic gradients across the mucosa. The addition of glucose to the luminal solution also had no effect either on p.d. or on short-circuit current, consistent with the lack of any demonstrable action of glucose on Na or water transport by colon (Powell & Malawer, 1968).

In several experiments, the effect of reducing the [NaCl] in the luminal

solution (osmolality being maintained constant by adding mannitol) or of substituting KCl or choline chloride for NaCl were examined. In untreated rats, with the p.d. relatively low (20 mV or less), the changes were always small and not consistent from animal to animal, observations comparable with those made previously when p.d. alone was observed (Edmonds & Marriott, 1968*b*). Probably in this situation the p.d. produced by active transport of ions by the tissue was obscured by diffusion p.d.s resulting from the ionic gradients produced across the epithelium when the composition of the luminal solution was changed. However, when the p.d. was high (> 30 mV), as in the aldosterone-treated rats, a consistent pattern was seen. Reduction in [NaCl] always produced a fall of p.d. and short-circuit current, tissue resistance being not significantly altered (Fig. 4). When [Cl] in the lumen was kept constant, K or choline being substituted for Na, the p.d. and short-circuit current were always markedly reduced but again tissue resistance was practically unchanged (Fig. 4). With Na absent from the lumen, the magnitude of p.d. and short-circuit current was similar whether or not the rats had been treated with aldosterone. Thus considerable alterations in the composition of the luminal solution does not affect tissue resistance nor in untreated rats cause more than small changes in p.d. or short-circuit current. When, however, the p.d. is elevated by aldosterone stimulation a clear dependence of p.d. and short-circuit current on [Na] becomes evident.

pH, osmolality, ouabain and cyanide. Altering the pH of the luminal solution by using phosphate or Tris buffer caused no changes over the range of pH 3·5–8·2 although outside this range, p.d. short-circuit current and tissue resistance tended to fall. Introduction of saline at pH 2, for example, caused a rapid fall of p.d. and short-circuit current. The effect was, however, reversible if the exposure to extreme pH was brief. Increasing the osmolality of the luminal solutions sufficiently to reverse water movement (Curran & Schwartz, 1960) by adding mannitol up to 500 m-osmole while keeping the [NaCl] constant was without influence on p.d. or short-circuit current. We found previously that ouabain in concentration 10^{-3} M applied directly *in vivo* to the serosal side of the epithelium produced a marked fall of p.d. while aldosterone applied in the same way in very low concentration produced a considerable rise of p.d. (Edmonds & Marriott, 1969). In some of the present experiments ouabain, 10^{-3} M, or aldosterone, 10^{-4} M, was contained in the luminal solution. However, neither produced any significant change of p.d. or short-circuit current even though exposure was from 1– 2 hr. Thus both aldosterone and ouabain need to be on the serosal side of the mucosa to produce their characteristic effects.

Cyanide is the only metabolic inhibitor which consistently reduces the

p.d. when placed in the lumen of the colon. Its action on the short-circuit current was examined in the present experiments, a solution of NaCN 30 mM–NaCl 120 mM being introduced into the lumen. A rapid fall of p.d. and short-circuit current occurred (Fig. 5) but in contrast to the effect of ischaemia, the p.d. change was relatively greater than that of current, indicating that tissue resistance had also fallen. Providing that exposure to cyanide was not prolonged, the p.d. and short-circuit current reverted to their former values when the solution was washed out of the lumen. There was, however, a delay of 20–30 min before recovery was complete.

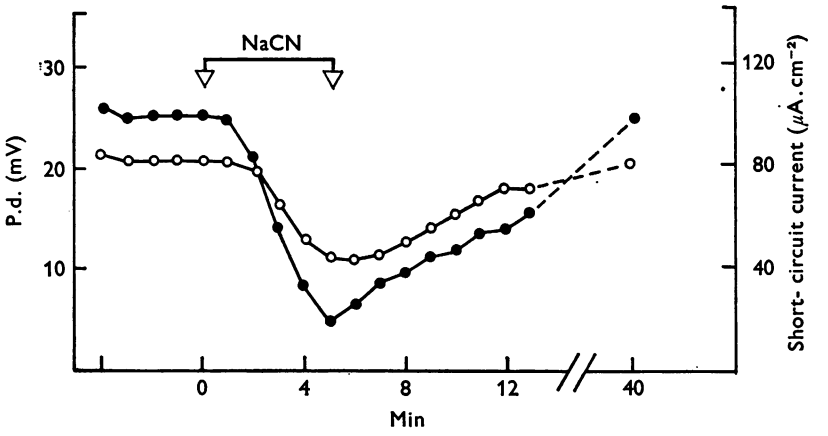

Fig. 5. Effect of a 5-min exposure of the mucosa to a luminal solution containing cyanide (NaCl 120 mM, NaCN 30 mM). The decline of p.d. was proportionately greater than that of short-circuit current indicating a fall in tissue resistance. Recovery began almost immediately after removal of the cyanide-containing solution from the lumen. ●, p.d.; ○, short-circuit current.

Short-circuit current and Na fluxes

The unidirectional fluxes of Na were measured both when the intestine was generating its spontaneous p.d. and when the p.d. across the tissue was reduced to zero by passing current (Table 1). In all experiments the flux of Na from lumen to the blood exceeded that into the lumen, Na absorption always occurring when 150 mM-NaCl was in the lumen. The Na absorption rate was considerably elevated by aldosterone treatment (Table 1), and when the preparation was short-circuited Na absorption rate rose in both groups of animals.

Short-circuit current and net Na transport. The p.d. was about twice as great in the aldosterone-treated as in the normal rats and there was a proportional rise in short-circuit current consistent with the observation that tissue resistance was not changed by aldosterone (Fig. 3). In normal

rats the Na actively transported accounted for an average of about 85% of the short-circuit current. The difference between the mean values of short-circuit current and active Na transport was not significant (Table 2). It seems likely, however, that a real difference did exist but was obscured by variation between rats for in all but two animals the short-circuit current exceeded the active Na transport. In the case of the aldosterone-treated rats short-circuit current corresponded closely with the amount of Na actively transported (Table 2).

TABLE 1. Na flux rates measured in the descending colon of normal and aldosterone-treated rats under open and short-circuit conditions, the lumen containing NaCl, 150 mM. The results are given as means ± 1 S.E.

		Open-circuit		
	No. of rats	Influx (J_i)	Efflux (J_o) (n-equiv min^{-1} cm^{-2})	Net (J_n)
Normal	8	145 ± 7·6	108 ± 10	37 ± 3·8
Aldosterone-treated	7	185 ± 20	87 ± 8·1	98 ± 15
		Short-circuit		
		Influx (J_i)	Efflux (J_o) (n-equiv min^{-1} cm^{-2})	Net (J_n)
Normal	8	172 ± 12	97 ± 13	76 ± 6·7
Aldosterone-treated	7	240 ± 25	66 ± 9·1	174 ± 17

TABLE 2. Comparison of net Na transport with short-circuit current in the descending colon of normal and aldosterone-treated rats, the lumen containing NaCl, 150 mM. The results are given as means ± 1 S.E.

	No. of rats	P.d. (mV)	Net Na flux rate (μA cm^{-2})	Short-circuit current (μA cm^{-2})
Normal	8	22 ± 1·1	120 ± 12	139 ± 8·3
Aldosterone-treated	7	41 ± 1·8	275 ± 27	259 ± 15

Changes in Na fluxes produced by altered p.d. If the efflux of Na were simply the result of diffusion from the extracellular fluid into the luminal solution, altering the p.d. across the mucosa either by aldosterone or by applying current should produce a predictable change in the Na efflux rate (Ussing, 1949). Thus if the Na efflux resulted entirely from passive diffusion into the lumen from the extracellular fluid, then in the aldosterone-treated rats, for example, the reduction of p.d. from 41 mV to zero by passing current would be expected to more than halve the efflux rate. Yet the observed fall was to only about 75% of the initial value. There was a similar discrepancy in the case of the normal rats. The findings suggested that only part of the Na efflux was due to simple diffusion and that a con-

siderable proportion was by some process not influenced by changes of p.d. This is consistent with some previous results (Curran & Schwartz, 1960; Edmonds, 1967*b*) in which the relationship between J_0 and luminal [Na] was investigated and showed that J_0 tended to fall as the luminal [Na] was reduced even when the p.d. was practically unchanged. Such an effect could

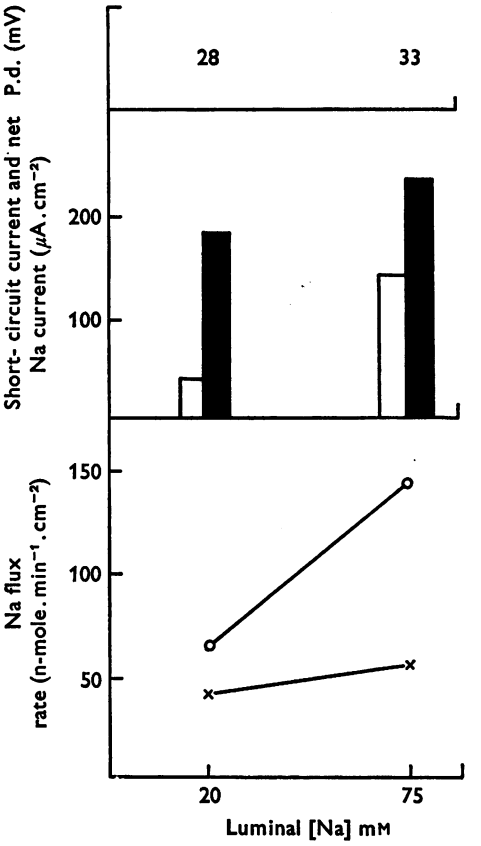

Fig. 6. Comparison of active Na absorption and short-circuit current in an aldosterone-treated rat when the lumen of the colon contained solutions of [NaCl] less than 150 mM. The Na unidirectional fluxes were determined while the tissue was short-circuited. The open columns indicate the Na net flux rate, the filled columns, the short-circuit current. ○, Na influx rate; ×, Na efflux rate.

occur if part of the Na efflux were due to exchange diffusion (Keynes, 1951). Then with only part of the Na efflux due to passive diffusion and so being influenced by p.d. changes, the changes of Na efflux rate produced by passage of short-circuit current would be less than expected.

Effect of reducing the luminal [*Na*]. Using toad colon, Cofré & Crabbé (1967) found that the short-circuit current could be attributed to the net Na absorption over a wide range of lumina [Na]. In several experiments of the present series, the effect of reducing the luminal [Na] on the short-circuit current and on net Na flux during periods of short-circuiting was examined. Unlike the situation in the toad colon, it was found that with reduced luminal [Na] there was a considerable discrepancy between the net Na movement and the short-circuit current, the latter always exceeding the former. The disagreement between the two variables became greater as the luminal [Na] was reduced. The results of a typical experiment are shown in Fig. 6. With 75 mM-NaCl in the lumen, the net Na movement was only about 60 % of the short-circuit current, while with 25 mM-NaCl in the lumen it accounted for less than 25 % of the short-circuit current. Thus rat colon differs from toad colon in that the short-circuit current appears to be attributable to active Na absorption only if the luminal [Na] is similar to that of extracellular fluid.

Sodium depletion or the presence of excess of Na retaining steroids either of endogenous or exogenous origin results both in man and other animals in a very low Na in the stool fluid (Milne, Muehrcke & Aird, 1957; Edmonds, 1967*a*; Charron, Leme, Wilson, Ing & Wrong, 1969; Richards, 1969). One possible explanation of this observation is that the critical luminal [Na] (that is the luminal [Na] at which Na influx and efflux rates are equal, $J_i = J_o$) is reduced by aldosterone treatment. Although in theory the most satisfactory way of determining the critical luminal [Na] is by direct measurement of Na flux rates at different luminal [Na] as discussed previously (Edmonds, 1967*b*), in practice, the considerable variability of the fluxes during experiments leads to difficulty. Several experiments of the type illustrated in Fig. 6 but without short-circuit current application were performed in an attempt to determine the critical luminal [Na] in several rats. The results suggested that the concentration was considerably less than 20 m-equiv/l. in the aldosterone-treated rats and considerably more than 20 m-equiv/l. in the normal rats. The variability meant, however, that the results were barely significant and accordingly an alternative method was employed in a further group of four normal and four aldosterone-treated rats.

A solution of [NaCl] 20 mM rendered isotonic with mannitol and containing phenol red was introduced into the lumen of a cannulated segment of colon and removed after a 20 min period. The change of [Na] was then determined. No significant change of volume was detectable over this short exposure period but the luminal [Na] did alter. In the case of normal rats it was found that a rise in [Na] always occurred, and after 20 min the [Na] was not less than 22 m-equiv/l. In contrast, when the rats had been

treated with aldosterone, it was found that the [Na] of the luminal solution had invariably fallen, a level of 15–17 m-equiv/l. usually being attained in 20 min. Although by this method, the critical luminal [Na] was not determined exactly, it did show that after aldosterone treatment its value was usually less than 15 m-equiv/l. while in the normal state its value was greater than 22 m-equiv/l. It can be concluded that aldosterone treatment lowers the critical luminal [Na] of the descending colon, the mucosal cells being able to remove Na from the lumen against the substantially greater [Na] gradient that in the untreated state.

DISCUSSION

The most important function of the colon, the conservation of Na and water, depends on the epithelial cells possessing a mechanism for absorbing Na from the gut lumen against the electro-chemical gradient. A number of epithelia have been shown to transport Na actively and in many of these it has been found using *in vitro* methods that the short-circuit current agrees well with the amount of Na actively transported (for example, with frog skin by Ussing & Zerahn, 1951; with toad bladder by Leaf, Anderson & Page, 1961). In the case of colonic epithelia, measurements have also been made using *in vitro* preparations. Coffré & Crabbé (1967) with toad colon showed that there was close agreement between the short-circuit current and active Na transport. However, in the case both of frog colon (Cooperstein & Hogben, 1959) and colon of Greek tortoise (Baillien & Schoffeniels, 1967) it was found that there was a discrepancy between these variables suggesting that active transport of other ions also contributed to the current.

The present study, employing an *in vivo* method developed to overcome the problems posed by the short life of isolated mammalian epithelia, demonstrated that for rat colon active Na transport accounted for nearly 90% of the short-circuit current under normal circumstances and with aldosterone stimulation it accounted for practically the entire short-circuit current. This was despite the fact that measurements were carried out with the solutions bathing the epithelium not completely at thermodynamic equilibrium. The experiments in which the luminal solution composition was altered did indicate, however, that small changes produced no significant effect on p.d. or short-circuit current. When the [Na] of the luminal solution differed considerably from that of extracellular fluid this was no longer true and changes of p.d. and short-circuit current were accompanied by a large discrepancy between the latter and the amount of Na actively transported. Since tissue resistance was unchanged over a wide range of p.d. values, it was clear that in the absence of large ionic gradients

across the mucosa, the magnitude of the p.d. depended predominantly on the amount of Na actively transported (Fig. 3).

There is evidence that other ions, for example, K and bicarbonate are also actively transported by mammalian colonic epithelium (Phillips & Code, 1966; Edmonds, 1967*c*) and they may account for the discrepancy in normal rats between the amount of Na actively transported and the short-circuit current. The influence of the other ions is small probably for two reasons. First, their effects tend to cancel as a result of secretion into the lumen of oppositely charged ions like K and bicarbonate. Probably, however, the most important factor is that the amounts of these ions actively transported are small compared with Na so that active Na transport is the major determining factor in the magnitude of the p.d.

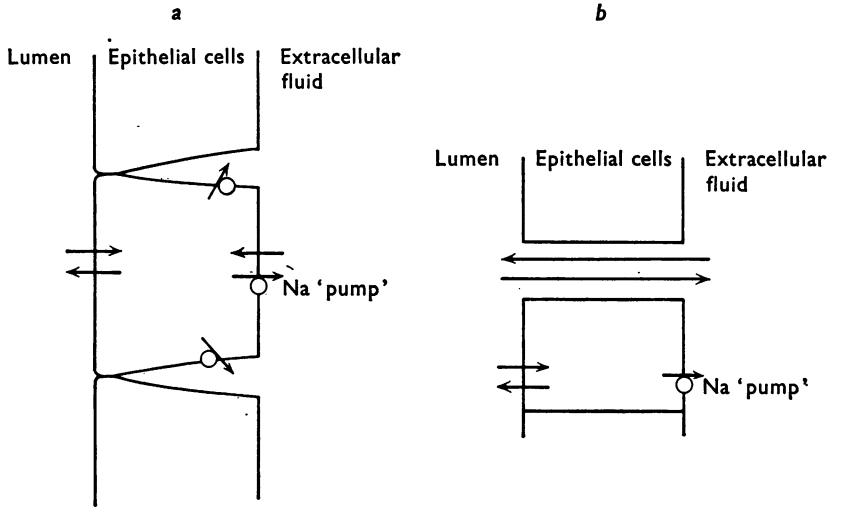

Fig. 7. Diagrammatic representation of Na transport across colonic epithelium. *a*, Na exchanges across the luminal face of the cell by passive processes while active Na extrusion occurs only from cell Na to the extracellular fluid. An action of aldosterone dependent on increase of Na permeability at the luminal face is incompatible in this model with the present finding of lowered critical luminal [Na]. *b*, model in which active and passive paths for Na are independent. The structural equivalents of the paths are not identified.

In other investigations (Edmonds & Marriott, 1968*a*, *b*; 1969) we found that various substances when applied to the serosal side could depress p.d. (for example, ouabain) or increase p.d. (for example, aldosterone) but when present in the lumen had little or no effect. The present results showing the lack of dependence of short-circuit current on most of the

changes in the luminal solution agreed with this earlier work. Cyanide was exceptional in being effective when in the lumen; it did, however, appear to be to some extent absorbed by the colon as stimulation of respiration was usually observed during the earlier part of the exposure. It is likely that the lack of action of most substances when in the lumen is explained by their failure to reach the Na pump. Thus the present findings suggest that the Na transport mechanism generating the p.d. and short-circuit current is separated from the lumen by a barrier which prevents most substances ever reaching it.

In the Na transporting epithelial cell, it is thought that transport results from Na entering the cell by one face (in the case of the intestinal cell, the face adjacent to the lumen) by processes not requiring energy expenditure but dependent on the electrical and [Na] gradients (Fig. 7*a*). Then through the basal and probably lateral plasma membranes, Na is extruded actively from the cell into the extracellular fluid. Two principal views have emerged about the way aldosterone exerts its effect on Na transport. They both regard the primary target for the hormone as being the cell nucleus with *m*-RNA and thus protein synthesis being influenced. However, while some believe that the proteins formed are enzymes concerned in the energetics of transport (as reviewed, for example by Edelman & Fimognari, 1968), others have suggested that the proteins are permeases which increase the permeability of a barrier limiting the rate of access of Na to the Na pump (Sharp & Leaf, 1966; Crabbé & de Weer, 1964). The first view is consistent with the present results since an influence on the energy supply could both stimulate Na absorption when the luminal [Na] is high and reduce the critical luminal [Na]. The permeability hypothesis, however, presents some difficulty. The present results suggested that some barrier did separate the luminal solution from the Na pump but although a change of permeability of this barrier could well increase active Na absorption when the luminal [Na] was high, as was observed, it would not be expected from the model (Fig. 7*a*) that the critical luminal [Na] would be lowered. Simply altering the permeability of the luminal face could not have this effect. But for rat descending colon, the present experiments showed that the critical luminal [Na] was lowered by aldosterone and similar findings were obtained in experiments on Na-depleted rats (Edmonds, 1967*b*). Clearly therefore the model of Fig. 7*a* cannot represent the situation in rat colon and be consistent with the permeability hypothesis. An alternative which still allows change of permeability to be the mode of action of aldosterone is shown in Fig. 7*b*. Here the pathways of Na transported actively and Na transported passively are independent. This seems to be an essential condition for the permeability hypothesis to be consistent with the finding of the reduced critical luminal [Na]. Thus the present results, although not

allowing discrimination between the two principal contending views on the action of aldosterone on Na transport, do place a restriction on the permeability hypothesis at least in regard to the colon.

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