Effects on fluid and Na$^+$ flux of varying luminal hydraulic resistance in rat colon *in vivo*

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1.   A new method of measuring fluid and ionic movements and the dehydrating power of the colon *in vivo* is described. A range of agarose gel cylinders, with calibrated hydraulic conductivities (L_p), were inserted into the lumen of the descending colon of anaesthetized rats. Fluxes of fluid, Na$^+$ and K$^+$ out of the gels were measured over a period of 60–110 min.

2.   Fluid absorption by the colon from 2·5 % agarose gels was not slower than from solution without gel. Fluid absorption was inhibited by 66 % when the agarose concentration was raised to 10 %. In contrast 2·5 % agarose gels caused a 73 % ($P < 0·001$) reduction in water flow from rat ileum.

3.   Increasing gel concentration to 10 % or above caused the absorbate from the gels to become hypertonic ($P < 0·001$).

4.   The measured suction pressure applied by the colonic hypertonic absorbate to the gels increased from $44 \pm 2·3$ cmH$_2$O ($n = 23$) with 2·5 % agarose gels to 6713 ± 960 cmH$_2$O ($n = 13$) with 15 % ($P < 0·001$).

5.   Deoxycholate (2 mM) produced a decrease in fluid and Na$^+$ absorption and reduced the suction pressure and power exerted by the colon.

Animals which make hard faeces, with a water content of less than 65 %, e.g. rats, rabbits, sheep and humans, do so by transporting fluid against the high hydraulic resistance imposed by the solid faecal mass (McKie, Powrie & Naftalin, 1990). The ability to move fluid against this hydraulic resistance is dependent upon the colon's capacity to generate a hypertonic absorbate (Bleakman & Naftalin, 1990; McKie, Goecke & Naftalin, 1991; Pedley & Naftalin, 1993). The hypertonic absorbate generates an osmotic gradient across the crypt wall which creates a high fluid tension within the crypt lumen. This tension sucks fluid from the faeces into the crypt lumen. Animals such as cattle, which cannot generate a hypertonic absorbate, are unable to make hard faeces and can only dehydrate faeces to *ca* 85 % water (Van Weerden, 1961; Maloiy, Taylor & Clemens, 1978).

Early studies in rats and humans which measured fluid transport *in vivo* (Parsons, 1956; Curran & Schwartz, 1960; Duthie, Watts, De Dombal & Galligher, 1964; Powell & Malawer, 1968; Billich & Levitan, 1969; Hawker, McKay & Turnberg, 1980) indicated that the colon can generate an absorbate tonicity of *ca* 500 mosmol kg^{-1}. The recent evidence for hypertonic absorption obtained using *in vitro*

preparations of isolated mucosa from rabbit and ovine colon has confirmed that colonic mucosa is capable of generating an absorbate *ca* 200 mosmol kg^{-1} hypertonic to plasma (Bleakman & Naftalin, 1990; McKie *et al.* 1991). We wished to confirm and extend these *in vitro* studies by observing the relationship *in vivo* between the hydraulic resistance of the colonic luminal contents and the rate of fluid absorption and tonicity of the fluid absorbate.

Hecker & Grovum (1975) estimated the rates of fluid absorption as a function of distance along the colon and of the changing fluid content of the faeces of sheep, pig and ox. In ovine colon, the fluid absorption diminished substantially in the distal colon as the faecal fluid content decreased from 90 to 60 %. With bovine colon, there was an abrupt decrease in fluid absorption in the caecum, as faecal water content fell from *ca* 97 to 85 % of the total weight, but faecal water content was virtually uniform along the remainder of the colon. Whether these changes in absorption rate were due to regional differences in the absorptive properties of the colon, or were a consequence of the varying hydraulic resistance of the faecal content as it consolidates, was not considered.

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We have attempted to answer these questions by measuring the net fluid and electrolyte flows across the colonic wall as a function of the hydraulic resistance of the luminal content. This has been achieved by monitoring the rates of fluid and ionic movements with a range of agarose gels of known hydraulic conductivity placed within the lumen of rat descending colon *in vivo*.

Some of the agarose gels were loaded with agents which either disrupt the mucosa or alter the osmotic pressure of the luminal contents. We chose deoxycholate (DOC) and polyethylene glycol 4000 (PEG 4000). DOC inhibits normal colonic fluid and electrolyte absorption by disruption of tight junctions, thereby increasing paracellular permeability (Freel, Hatch, Earnest & Goldner, 1983a, b; Bleakman & Naftalin, 1990). It also stimulates adenylate cyclase activity, thereby raising K^+ conductance of the apical membrane and thus inducing active K^+ secretion into the colonic lumen (Conley, Coyne, Bonnoris, Chung & Schoenfeld, 1976; Wanitschke, Nell, Rummel & Specht, 1977; Freel *et al.* 1983a, b; Smith & McCabe, 1984). PEG 4000 (10 mM) has a high osmotic coefficient and exerts an osmotic pressure of *ca* 15 mosmol kg^{-1} (Naftalin & Tripathi, 1985).

METHODS

Where error margins are given they denote the s.e.m. Where significance values are given, they are derived using Student's *t* test.

Preparation of agarose gels

Agarose type II-A (Sigma Chemical Co.) was chosen as our gelling agent because of its high strength and biological inertness. The agarose content of the gels ranged from 2·5 to 15%, i.e. 2·5 to 15 g per 100 ml of vehicle solution (140 mM NaCl, 5 mM KCl). The agarose solutions were heated under pressure for about 10 min at 110 °C. The agarose solution was then cast in glass tubes of uniform internal diameter (6 mm). The cooled gels were expressed from their moulds, cut into 2 cm lengths and weighed, prior to insertion into the colonic lumen. Where

deoxycholate (DOC, 2 mM; Sigma) and/or polyethylene glycol (PEG 4000; MW, 4000; 10 mM; BDH Chemicals Ltd, Poole, UK) were used, they were dissolved in the vehicle solution prior to addition of the agarose and heating.

Measurement of hydraulic conductance of agarose gels

The hydraulic conductivity of the agarose gels (expressed as L_p (cm^3 cm^{-2} s^{-1} cmH$_2$O^{-1}, equivalent to cm s^{-1} cmH$_2$O^{-1}) was measured by monitoring pressure-induced water flow through gels of varying agarose concentration and thickness. The gels were contained within 20 ml plastic syringes and supported on a sintered glass disc covered with filter paper. Fluid flow was determined by weighing the eluant from the syringes after periods of sustained pressure (*ca* 400 cmH$_2$O, monitored with a mercury manometer), varying from 30 min to 72 h.

Variation of L_p with agarose concentration of the gel

The effect of varying the agarose concentration on the L_p of 5 mm thick gels are shown Fig. 1. The L_p decreased as a monoexponential function of agarose concentration over the entire range tested (2·5 to 15%) fitted by exponential regression analysis ($P < 0.001$). The L_p decreased from 4×10^{-7} to 5×10^{-10} cm s^{-1} cmH$_2$O^{-1} over this range.

Variation of L_p with agarose gel thickness at constant agarose concentration

The effect of varying the thickness of agarose gels of 2·5, 5 and 7·5%, from 2·5 to 15 mm, on the L_p are shown in Fig. 2. There were monoexponential decreases in the values of L_p of the agarose gels over the range of thicknesses tested. Decreasing the gel thickness from 6 to 2·5 mm, the maximal extent of the changes in gel diameter observed during gel dehydration within the rat colonic lumen (see below), had only a small effect on the L_p of the gels.

Insertion of agarose gels into the rat colon

Wistar rats weighing between 100 and 150 g were anaesthetized by intraperitoneal injection of pentobarbitone sodium BP (120 mg kg^{-1}; May and Baker Ltd, Dagenham, UK). The descending colon was opened by an incision and faeces flushed from the lumen. The gels were inserted into the lumen proximal to the incision. A ligature was placed at least 1 cm distal to the gels to secure them.
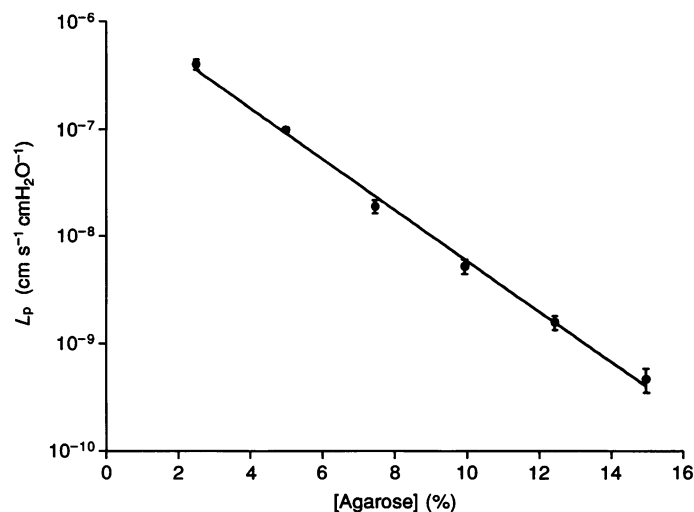

Figure 1. The effects of increasing the concentration of agarose on the L_p of the gel
The L_p decreases as a monoexponential function of agarose gel concentration over the range tested, 2·5 to 15%. Line fitted by monoexponential regression $y = A\exp(Bx)$, where A and B are constants and x the independent variable
($y = 1·364 \pm 0·039 \times 10^{-6}\exp(-0·547 \pm 0·008x)$, $r = 0·99$). At each agarose concentration, n was between 3 and 8 and the error bars represent the s.e.m.

Monitoring the changes in fluid and ionic composition of the agarose gels

After incubation, the gels were removed from the lumen and reweighed to determine fluid loss. The macerated gels were extracted overnight in 0·1 M HCl. The amounts of Na^+ and K^+ in the HCl extracts were measured using an Instrumentation Laboratory 943 automated flame photometer. The rates of fluid and Na^+ loss, and K^+ loss or gain, were expressed as microlitres (fluid) or micromoles (ions) per square centimetre surface area of the gel per hour of exposure.

The absorbate tonicity was determined by the ratio of amount of Na^+ ($\mu mol\ cm^{-2}\ h^{-1}$) to volume of fluid absorbate ($cm^{-3}\ cm^{-2}\ h^{-1}$). Hypertonicity (in cmH_2O) of the absorbate above plasma (which has an osmolality of 300 mosmol kg^{-1}) was calculated by:

$$Hypertonicity = [(Na^+\ absorbate\ tonicity \times 2) - 300] \times 26,$$

where 1 mosmol kg^{-1} is equivalent to 26 cmH_2O. We have ignored the contribution of K^+ flux to the absorbate tonicity as this is relatively small and its contribution to paracellular flux is uncertain.

Estimation of the suction pressure on the agarose gels and power output of colon

The suction pressure, ΔP (cmH_2O), across a hydraulic conductance can be characterized by $\Delta P = J_v / L_p$, where J_v is fluid flux ($cm^3\ cm^{-2}\ s^{-1}$) and L_p is measured in centimetres per second per centimetre water (the measured gel hydraulic conductivity as described above). The external power generated per square centimetre of colon exposed to gel per second was calculated (in ergs) from:

$$Power\ (ergs) = \Delta P\ (cmH_2O) \times vol.\ of\ fluid\ absorbed\ (cm^3\ s^{-1}),$$

and expressed in picowatts per square centimetre (where $10^7\ ergs\ s^{-1} = 1\ W$).

Agarose gels in the rat ileum and the method of their insertion

Agarose gels for the ileum were made in the same way as for the colon but with the addition of 15 mM glucose to the vehicle solution and were cast in tubes of a smaller internal diameter (4 mm). An incision was made in the terminal ileum about 10 cm proximal to the caecum. Luminal contents were removed and gels gently moved along the ileum in a proximal direction until about 15 cm from the incision. The fluid and electrolyte movements were monitored in the same ways as described for colonic gels.

Method of monitoring fluid transport from colonic and ileal lumen in the absence of agarose gel

The methods for estimating fluid absorption in rat colon *in vivo* in the absence of agarose gels were the same as previously reported (Mendizabal & Naftalin, 1992a, b). A known volume of saline (0·3 ml (cm length)$^{-1}$) containing Dextran Blue (2 MDa; 1 mg (100 ml)$^{-1}$) was injected into a segment of colon and sealed at either end by a ligature. After 2 h the fluid was removed from the lumen and the volume loss estimated from the concentration changes of the volume marker, using spectrophotometric assays.

For measurements in the ileum, segments of the terminal ileum about 6–9 cm in length were ligatured at one end, filled with a known volume of 140 mM NaCl, 5 mM KCl and 15 mM glucose solution and ligatured at the other end. After 60 min, the fluid was removed, weighed and the concentrations of Na^+ and K^+ measured as described above.

RESULTS

Time course of fluid and electrolyte absorption from 2·5% agarose gels

Fluid and Na^+ uptake rates from 2·5% agarose gels inserted into the colonic lumen were in a steady state from 30 min incubation and continued at a constant rate for at least a further 80 min (Fig. 3). In the subsequent experiments we have monitored the rates of fluid, Na^+ and K^+ fluxes from gels over periods varying from 85 to 100 min.

Effects of various agarose concentrations on fluid and Na^+ fluxes

The effects of various agarose concentrations on fluid and Na^+ absorption from the gels are shown in Figs 4 and 5. The rate observed in the absence of gel was

Figure 2. The effects of increasing the gel thickness on the L_p of the gel at constant agarose concentrations

Agarose concentrations: ●, 2·5%; ○, 5%; ■, 7·5%. There were monoexponential decreases in the values of L_p of the gels over the range of gel thickness tested. Lines fi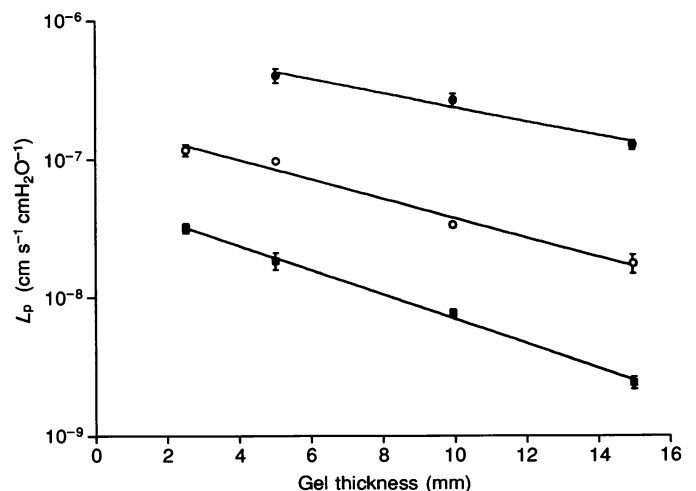tted by monoexponential regression $y = A\exp(Bx)$ (for 2·5%, $y = 7·5 \pm 1·0 \times 10^{-7}\exp(-0·117 \pm 0·019x)$, $r = 0·985$; for 5%, $y = 1·85 \pm 0·23 \times 10^{-7}\exp(-0·161 \pm 0·029x)$, $r = 0·98$; and for 7·5%, $y = 5·24 \pm 0·20 \times 10^{-8}\exp(-0·20 \pm 0·01x)$, $r = 0·99$). At each point n was between 2 and 8, and the error bars represent the s.e.m.
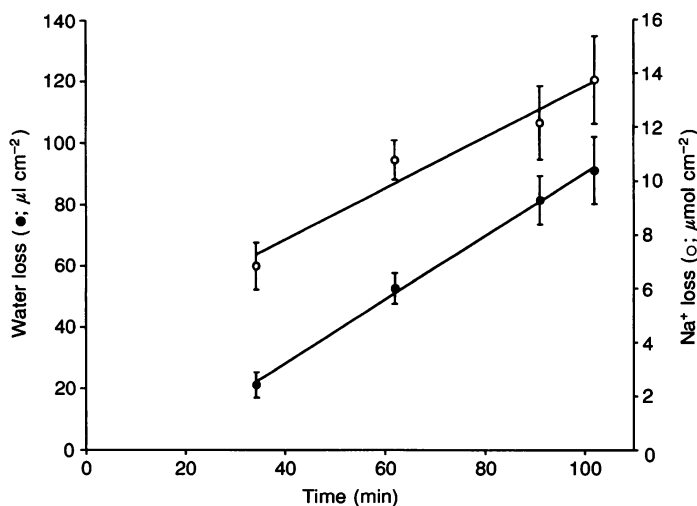
Figure 3. The time course of fluid and net Na⁺ absorption from 2·5% agarose gels placed in rat descending colon

Fluid and Na⁺ absorption from the gels was in a steady state from 30 min incubation and continued at a constant rate for at least a further 80 min. Lines fitted by linear regression $y = A + Bx$ (for water flux $y = -12·75 \pm 2·28 + 1·02 \pm 0·03x$, $r = 0·99$; and for Na⁺ flux $y = 4·04 \pm 1·12 + 0·09 \pm 0·01x$, $r = 0·98$). At each time point n was between 4 and 9, and the error bars represent the S.E.M.
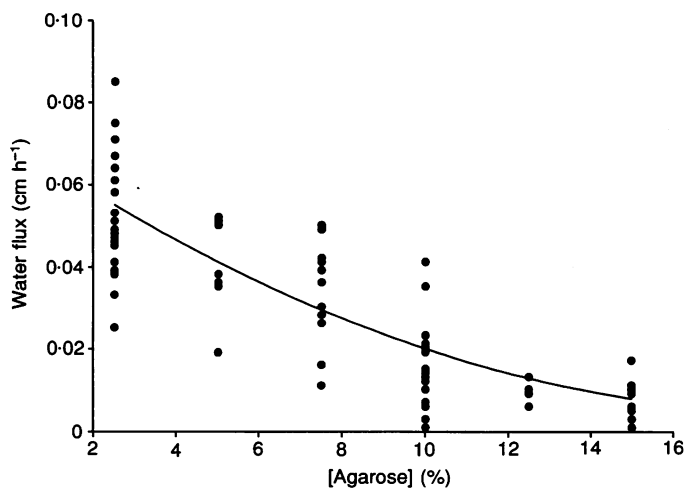

Figure 4. The effects of varying the concentration of agarose on the water absorption by the colon

There was a progressive decrease in the rate of fluid absorption as the agarose concentration is increased ($P < 0·001$). Line fitted by second-order regression $y = A + Bx + Cx^2$ (for water flux $A = 0·071 \pm 0·004$, $B = -0·007 \pm 0·001$ and $C = 0·00018 \pm 0·00008$; $r = 0·89$. Each point represents a single gel.
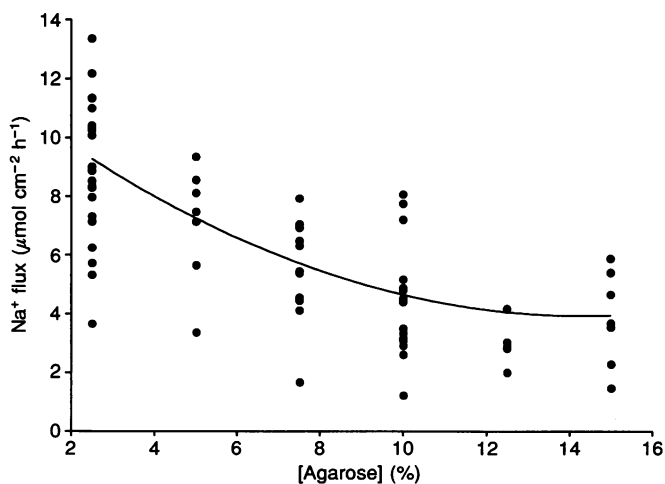

Figure 5. The effects of varying agarose concentration on Na⁺ absorption from the luminal gels within rat descending colon

A significant decrease in Na⁺ absorption on increasing gel concentration from 2·2 to 12·5%) ($P < 0·001$) was observed. Na⁺ flux line fitted by second order polynomial regression ($y = A + Bx + Cx^2$; $A = 11·82 \pm 0·74$; $B = -1·11 \pm 0·22$; $C = 0·039 \pm 0·013$; $r = 0·752$). It should be noted that there is a significant rate of Na⁺ absorption from 15% gels when water absorption is near to zero (cf. Fig. 4). Each point of the data represents the Na⁺ flux from a single gel.

Figure 6. The residual concentrations of Na$^+$ and K$^+$ within gels of different agarose concentrations after incubation in the colon for between 85 and 100 min

●, Na$^+$; ○, K$^+$. The initial concentrations of Na$^+$ and K$^+$ within the gels were 140 and 5 mM respectively. There was no significant change in residual K$^+$ or Na$^+$ as the agarose concentration was increased from 2·5 to 15%. Lines fitted by linear regression, $y = A + Bx$ (for [Na$^+$], $y = 84·55 \pm 3·29 + 1·26 \pm 0·76x$, $r = 0·43$; and for [K$^+$], $y = 13·87 \pm 1·11 - 0·42 \pm 0·25x$, $r = 0·35$). Each point of the data represents a reading from a single gel.
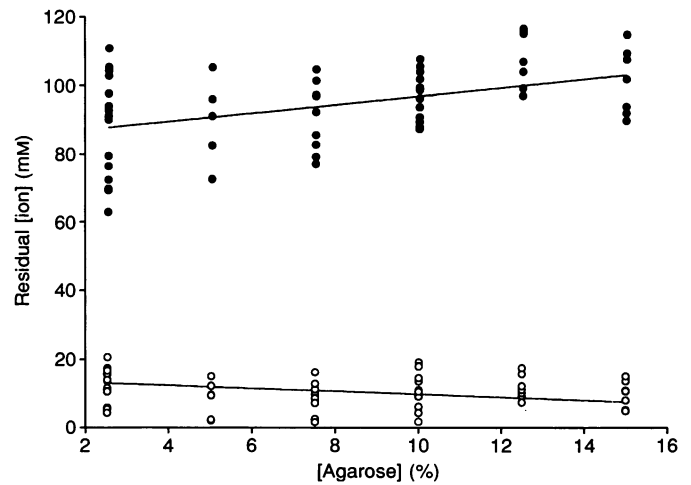
$50 \pm 2·9 \, \mu$l cm^{-2} h^{-1} ($n = 17$). With 2·5% agarose, fluid absorption was $53 \pm 5 \, \mu$l cm^{-2} h^{-1} ($n = 21$). There was a progressive decrease in both the rate of fluid and Na$^+$ absorption as the agarose concentration increased ($P < 0·001$). However, even when fluid absorption was reduced almost to zero at high gel concentrations ($> 12·5$%), a significant net Na$^+$ absorption was still observed: $2·86 \pm 0·35$ ($n = 5$) and $3·72 \pm 0·44 \, \mu$l cm^{-2} h^{-1} ($n = 13$) at 12·5 and 15% gel concentrations, respectively ($P < 0·001$), indicating that the absorbate concentration increased at higher agarose concentrations. However, a significant decrease in Na$^+$ flux ($P < 0·001$) from the gel was noted as agarose concentration was increased.

The residual concentrations of Na$^+$ and K$^+$ within the gels after incubation for 85–100 min in the colon are shown in Fig. 6. The concentration of Na$^+$ fell from 140 mM at zero time to an average for all gels of $95·3 \pm 1·6$ mM ($n = 57$; $P < 0·001$), whereas K$^+$ concentration rose from 5 mM at zero time to an average of $10·8 \pm 0·9$ mM ($n = 66$; $P < 0·001$). These findings indicate that there was both

hypertonic Na$^+$ absorption from the gels and K$^+$ secretion into the lumen. There is no significant overall correlation between the residual concentrations of either Na$^+$ or K$^+$ with gel concentration.

Effects of various agarose concentrations on absorbate concentration and suction pressure on the gel

The absorbate tonicity, expressed as Na$^+$ concentration (mM), in the fluid absorbate from the gels is shown plotted in relation to gel concentration in Fig. 7. A second-order polynomial is fitted to the data ($r = 0·797$). With gel concentrations in the range 0–5% the [Na$^+$] in the absorbate was only slightly higher than that of the bathing solution (140 mM): [Na$^+$] = $174·5 \pm 5·7$ mM ($n = 37$; $P < 0·001$). However, as the gel concentration rose above 10%, a dramatic increase in [Na$^+$] was seen ($P < 0·001$); at 15% the absorbate [Na$^+$] was 436 ± 37 mM ($n = 11$), which gives a tonicity of the absorbate almost 3-fold higher than that of plasma.

Figure 7. The calculated Na$^+$ tonicity (mM) of the absorbate as the agarose concentration increases

As the agarose concentration increases, the hyper-tonicity of the absorbate increases ($P < 0·001$). The line is a second-order polynomial regression line of the tonicity (y) *versus* agarose percentage concentration (x). The regression coefficients of the line through all the points are: $A = 187 \pm 25·4$; $B = -11·55 \pm 7·64$; $C = 1·89 \pm 0·46$; $r = 0·797$.
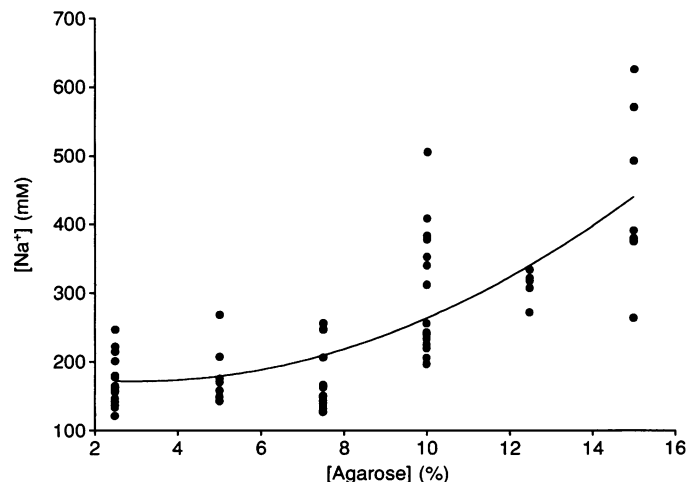
The suction pressure on the gel (estimated as described in Methods) increased exponentially as the agarose concentration was raised, from 44 ± 2.3 cmH$_2$O with 2·5% agarose gels ($n = 23$) to 6713 ± 960 cmH$_2$O ($n = 13$) with 15% agarose ($P < 0.001$; Fig. 8). The hypertonicity of the absorbate estimated from the [Na$^+$] of the gel absorbates alone is plotted in the same scattergraph. At low gel concentrations the suction pressure is only 2·5% of the theoretical maximum which could be exerted by the osmotic pressure of the absorbate; however, as the gel concentration rises to 15% agarose, the observed suction pressure rises to ca 45% of the maximal theoretical value calculated from the absorbate tonicity (14915 ± 1941 cmH$_2$O, $n = 11$).

Power output of the descending colon

The external power generated by the colon is shown as a function of agarose concentration over the range 2·5–12·5% in Fig. 10. The error on the 15% agarose gel was too large to be included. The power output exerted on luminal gels increased from 72 ± 7 pW cm^{-2} ($n = 31$) with 2·5% gels to maximum output of 649 ± 113 pW cm^{-2} ($n = 5$) with 12·5% gels ($P < 0.001$).

Ileal fluxes of fluid, Na$^+$ and K$^+$ from agarose gels

Fluid absorption from the lumen of small intestine in the absence of gel was approximately 1·4-fold faster than from colon with equivalent fluid content, i.e. $69 \pm 2.5 \mu$l cm^{-2} h^{-1} ($n = 13$). However, even the modest hydraulic resistance caused by 2·5% agarose gels (4×10^{-7} cm s^{-1} cmH$_2$O^{-1}) significantly reduced fluid movement by ca 73%, to $19 \pm 1 \mu$l cm^{-2} h^{-1} ($n = 22$; $P < 0.001$). In the colon, there was no reduction of fluid transport with this concentration of agarose gel, compared with absorption from fluid media (54 ± 4, $n = 17$, vs. $50 \pm 2.9 \mu$l cm^{-2} h^{-1}, $n = 25$; Fig. 9). The suction pressure exerted by ileum was 13 ± 2 cmH$_2$O and the power exerted on the gel 7 ± 1 pW cm^{-2}, which is 10% of that exerted by colon on 2·5% gels (Fig. 10).

The ileal Na$^+$ fluxes from 2·5% agarose gels were $2.47 \pm 0.21 \mu$mol cm^{-2} h^{-1} ($n = 25$), giving an absorbate tonicity of 130 ± 13 mM (compared to ca 173 ± 6 mM for 2·5% agarose in the colon). The ileal absorbate [Na$^+$] was not significantly different from isotonicity (140 mM). The K$^+$ flux into 2·5% agarose gels in the ileum was $0.22 \pm 0.03 \mu$mol cm^{-2} h^{-1} ($n = 25$).
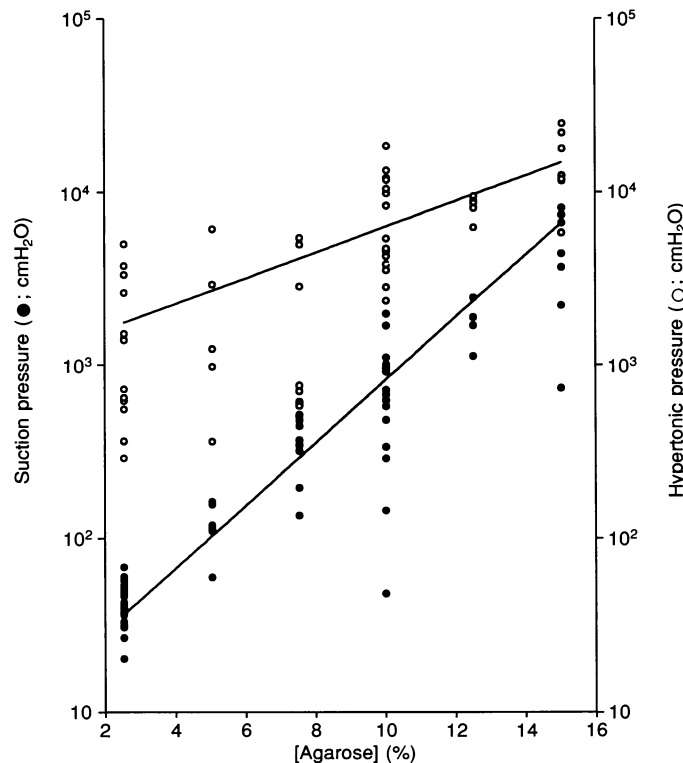

Figure 8. Effect of varying gel agarose content on the suction pressure and the hypertonicity of the absorbate (absorbate hypertonicity (cmH$_2$O) = (absorbate [Na$^+$] $\times$ 2 $-$ 300) $\times$ 26 (cmH$_2$O) (see Methods)

The lines through the data are the least-squares monoexponential regression lines $y = A\exp(Bx)$. For hypertonic pressure $A = 1132 \pm 186$; $B = 0.172 \pm 0.016$; $r = 0.907$. For suction pressure $A = 12.7 \pm 6.8$; $B = 0.417 \pm 0.036$; and $r = 0.968$.

Figure 9. Calculated power from the colon that is supplied to dehydrate gels of increasing agarose concentration

As the agarose concentration increases, the power supplied to dehydrate the gels increases to a maximum of *ca* 650 pW cm^{-2}. Line fitted by third-order polynomial regression, $A + Bx + Cx^2 + dx^3$; $A = 427 \pm 43$; $B = -260 \pm 28$; $C = 53.57 \pm 4.98$; $D = -2.50 \pm 0.25$; $r = 0.9999$. n for each gel concentration is shown beside the mean and S.E.M.
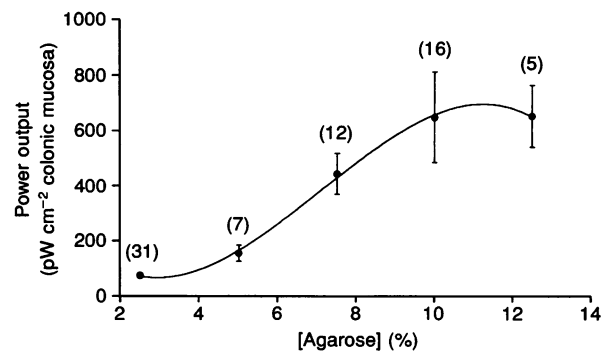
Effects of deoxycholate on colonic fluid and electrolyte fluxes in agarose gels

At concentrations of DOC ≥ 5 mM, net fluid and Na$^+$ absorption from 2·5% agarose gels was prevented and K$^+$ flux into the gel was increased (P. S. Zammit & R. J. Naftalin, unpublished observations). At a DOC concentration of 2 mM, there was a significant decrease in net fluid from $54 \pm 4 \mu l$ cm^{-2} h^{-1} in controls ($n = 21$) to $30 \pm 8 \mu l$ cm^{-2} h^{-1} ($P < 0.01$) and an increase in K$^+$ secretion from $0.48 \pm 0.08 \mu$mol cm^{-2} h^{-1} in controls ($n = 11$) to $1.11 \pm 0.18 \mu$mol cm^{-2} h^{-1} ($n = 22$; $P < 0.01$). The residual [Na$^+$] increased within the gels significantly above controls from 82.59 ± 5.46 mM ($n = 13$) to 101.55 ± 2.13 mM with DOC (2 mM; $n = 9$; $P < 0.05$) and the residual [K$^+$] increased from a control value of 11.93 ± 1.44 ($n = 14$) to 20.57 ± 1.1 mM ($n = 11$; $P < 0.001$). The suction pressure on 2·5% gel was significantly reduced from a control value of 38 ± 5 ($n = 21$) to 21 ± 6 cmH$_2$O ($n = 8$; $P < 0.01$) with 2 mM DOC.

DISCUSSION

Effects of luminal hydraulic resistance on fluid absorption in the colon

The role of extracellular hypertonicity in generating the osmotic pressure to induce directional water flow across epithelia was proposed by Curran & McIntosh (1962) and refined by Diamond & Bossert (1967). However, in order to

dehydrate faeces or a loose hydrogel, such as used in this study, the osmotic pressure has to be transduced to a hydrostatic suction pressure which can be propagated some distance from the epithelial membrane surface into the semi-solid luminal contents of the colon. The mechanism whereby the colon transduces the large osmotic pressure of the pericryptal fluid surrounding colonic crypts into a large hydraulic suction pressure without preventing fluid reflux via wide paracellular channels has been discussed previously (Bleakman & Naftalin, 1990; McKie *et al.* 1991; Pedley & Naftalin, 1993). The results in this paper show that the mechanism proposed applies also to the *in vivo* condition and for the first time quantifies the colonic dehydrating force and power.

Apart from the observations of Hecker & Grovum (1975), showing indirectly that there may be an effect of the consolidation of faecal contents on the rate of fluid absorption from the colon of sheep, pigs and cattle, there has been no previous published work quantifying the effects of hydraulic resistance on fluid absorption from the colonic lumen. Our results clearly demonstrate that as the hydraulic resistance of the luminal contents increases, the fluid reabsorption decreases (Fig. 4). As with an analogous electrical circuit, series addition of an external (luminal) hydraulic resistance equal to the hydraulic resistance of the battery (tissue) reduces the current (fluid) across the circuit by 50%. The agarose concentration required to give a 50%

Figure 10. The differences in colonic and ileal absorption of water and the effects of the hydraulic resistance imposed by a 2·5% agarose gel

In the colon (□), the rate of water absorption is unaffected by a 2·5% agarose gel, while in the ileum (▨), absorption is reduced by *ca* 73% by the gel ($P < 0.001$). The values are means $\pm$ S.E.M. and in all cases for ileal fluid $n = 13$ and for colonic fluid $n = 17$; for 2·5% agarose gels with ileum $n = 22$ and with colon $n = 25$.
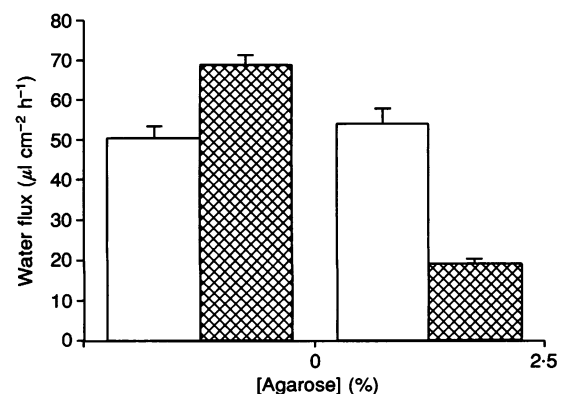
($27\ \mu l\ cm^{-2}\ h^{-1}$) decrease in fluid absorption was 8.5% w/v (from the regression line in Fig. 4). Assuming that the hydraulic resistance of the colon is unaltered by the imposition of a substantial luminal hydraulic resistance, then it can be deduced that the average fluid conductance across rat colon *in vivo* is equivalent to an L_p of $1.3 \times 10^{-8}\ cm\ s^{-1}\ cmH_2O^{-1}$. This estimate of tissue hydraulic conductance *in vivo* is 15-fold lower than that for rabbit colon *in vitro* ($2 \times 10^{-7}\ cm\ s^{-1}\ cmH_2O^{-1}$; Bleakman & Naftalin, 1990). In rat ileum, fluid absorption was reduced by 73% with a 2.5% agarose gel, whereas in colon a 2.5% gel had no significant effect on absorptive flow (Fig. 10). Thus, rat colon has a *ca* 100-fold greater capacity to dehydrate against a hydraulic resistance than rat ileum because the hydraulic conductance of rat ileum is much higher ($1.36 \times 10^{-6}\ cm\ s^{-1}\ cmH_2O^{-1}$).

This first-order approach to estimating the hydraulic resistance of the colonic mucosa is likely to be an over-simplification. As the absorbate tonicity increased when the agarose concentration was increased, this implies that there was a reduction in the tissue paracellular hydraulic and ionic conductance at higher gel concentrations. This gearing of the dehydrating mechanism to the load is a practical way of rapidly reducing the water content of fluid faeces when the ratio of fluid to solids exceeds 15; however, as the luminal resistance increases, the fluid tension within the crypt lumen reduces the spaces between the colonocytes and thereby increases the paracellular hydraulic resistance permitting fluid to be extracted down to a faecal fluid/solid ratio of *ca* 2:1.

The large difference between the hydraulic conductivity of rabbit colon *in vitro* (Bleakman & Naftalin, 1990) and rat colon *in vivo* (this study) can be ascribed mainly to the dehydrating effects of agarose gels on the colonic lumen. The L_p of rabbit and rat colon is not uniformly distributed. The paracellular hydraulic conductance is the predominant conductance for positive luminal pressure and colloid osmotic pressure-induced water movement, whereas the transcryptal route, which has a much lower hydraulic conductance of *ca* $1-2 \times 10^{-10}\ cm\ s^{-1}\ cmH_2O^{-1}$, accounts for the osmotic pressure-induced water flow by NaCl. The results in this paper indicate that when the luminal hydraulic resistance is raised the paracellular hydraulic conductance of rat colon approaches that of the transcellular route.

Effects of luminal hydraulic resistance on the colonic absorbate tonicity

Our previous work (McKie *et al.* 1991) showed that a very large pressure (5–10 atm, i.e. 5000–$10000\ cmH_2O$) was required to dehydrate faeces to the levels obtained in hard faeces, i.e. < 70% water, in the 4–5 h period available for its formation within the colonic lumen. We have also shown that the colonic absorbate has a tonicity which is

theoretically capable of generating a large suction pressure, that the interstitial fluid *in vitro* is very hypertonic, *ca* $1500\ mosmol\ kg^{-1}$, and that in the normal absorptive mode, there is a rapid inflow of fluid into the crypt lumen of rat and ovine colon (Bleakman & Naftalin, 1990; Naftalin & Pedley, 1990; McKie *et al.* 1991; Pedley & Naftalin, 1993).

On increasing the agarose concentration of the luminal gels, the net fluid absorption was decreased proportionally more than the rate of net Na^+ absorption (see Figs 4 and 5). There was an exponential increase in the absorbate concentration as the agarose concentration was raised (Fig. 8). This is also displayed in Fig. 8 as the theoretical osmotic pressure exerted by the hypertonic absorbate on dialysis against isotonic plasma, i.e. ideal osmotic pressure. The suction pressures on the gels required to account for the observed rates of gel dehydration also increase exponentially with agarose concentration (Fig. 8). Rat descending colon can develop a suction pressure of > 5 atm (or $5000\ cmH_2O$). Although a very high suction pressure, *ca* -4 atm (i.e. $4000\ cmH_2O$ below atmospheric pressure), it is still only 45% of the theoretical maximum which could be developed by the hypertonic absorbate. These large negative pressures are similar to the fluid tension observed in tree roots (Pickard, 1981) or octopus suckers (Smith, 1991).

Effects of luminal hydraulic resistance on water and ionic absorption in the ileum

The failure of rat ileum to dehydrate agarose gels of 2.5% to the same extent as colon can be related to studies which have shown that the ileal hydraulic conductance is raised asymmetrically when a serosa–mucosa hydrostatic pressure gradient is applied (Loeschke, Bentzel & Csaky, 1970; Bindslev, Tormey & Wright, 1974; Naftalin & Tripathi, 1985). The high rate of paracellular leakage of fluid and solute in ileum explains why a prolonged residence time of chyme within the small intestine does not reduce the hydration of luminal contents below 90% water, whereas prolonged residence of faeces in the colon produces constipation, with faeces having a water content of *ca* 60%. Similarly, the raised paracellular solute and hydraulic permeability seen in inflammatory bowel disease and in model colitis systems also results in the inefficient dehydration of faeces (Duthie *et al.* 1964; Harris & Shield, 1970; Hawker *et al.* 1980; Krugliak *et al.* 1989).

Effects of luminal hydraulic resistance on Na^+ flux in the colon

Previous work has demonstrated that the Na^+ concentration of faeces is low, indicating that it is actively absorbed (reviewed by Powell, 1979). Our results confirm these observations, as the steady-state concentration of Na^+ in the gels after incubation within the colon was below the initial concentration, which was equal to that of plasma (140 mM). However, our results demonstrate that Na^+

absorption is also correlated with the hydraulic resistance of the luminal contents. When the hydraulic resistance of the luminal contents was raised, the net Na^+ absorption was decreased and thus the residual Na^+ concentration within the luminal gels was increased.

Effects of DOC on fluid and electrolyte movements in the colon

The bile salt DOC (2 mM) causes a reduction in fluid flux but increases K^+ flux into the lumen significantly and therefore raises the residual K^+ concentration within the gel. These data indicate that DOC stimulates an active transcellular secretory process, such as is observed with other laxatives, e.g. bisacodil or phenolphthalein, which raise cyclic AMP within colonic mucosa and increase K^+ conductance across the apical membrane (Conley *et al.* 1976; Wanitschke *et al.* 1977; Freel *et al.* 1983*a*, *b*; Smith & McCabe, 1984). DOC reduces the colonic suction capacity, as demonstrated by the rise in the ratio of absorbate hypertonicity/gel suction pressure.

Effects of DOC on the external suction power output by the colon

The external power output increases from 72 ± 7 pW cm^{-2} with a 2.5% agarose gel to 649 ± 113 pW cm^{-2} with 12.5%. This increase in power is likely to be due to the decreased paracellular conductance resulting from the reaction of the tissue to the suction pressure exerted on the mucosa. There is a loss of external suction power when DOC is present within 2.5% agarose gels compared with controls, falling from 57 ± 9 to 7 ± 1 pW cm^{-2} ($P < 0.001$). The results are an indication that the dehydrating capacity and power of the colon depend on the tightness of the intercellular junctions and that breakdown of these will severely impair the ability to generate hard faeces.

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