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Influence of serosal hydrostatic pressure on net water and electrolyte transport across the isolated rat colonic mucosa exposed to different secretagogues

U. Karbach [1] **and R. Wanitschke** [2]

[1] Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, D-6650 Homburg/Saar
[2] I. Medizinische Klinik und Poliklinik der Universitätsklinik, D-6500 Mainz, Federal Republic of Germany

Summary. 1. The influence of 2 cm and 7 cm hydrostatic pressure applied upon the serosal side on net water and electrolyte transport and paracellular permeability was investigated in everted sacs of stripped rat colon mucosa exposed to different secretagogues.

2. A 2 cm pressure abolished net fluid absorption in the presence of deoxycholic acid, bisacodyl, ethacrynic acid and rhein and reduced absorption in the tissue pretreated with cholera toxin.

3. The paracellular permeability was increased by deoxycholic acid, bisacodyl and ethacrynic acid and diminished under the influence of rhein and cholera toxin.

4. At a pressure of 7 cm H_2O fluid movement was directed toward the mucosal side parallel to the increase of the paracellular permeability. The fluid appearing at the mucosal side was isotonic in the presence of deoxycholic acid, ethacrynic acid and rhein but hypotonic when the tissue was pretreated with cholera toxin.

5. From the pressure-induced net water flow and the composition of the transferred fluid secretagogues acting predominantly on paracellular pathway can be distinguished from secretagogues acting on basis of other mechanisms.

Key words: Rat colon – Hydrostatic pressure – Secretagogues – Sodium and chloride transport – Water transport

Introduction

Two mechanisms have been proposed to explain secretion of water and electrolytes in the intestine: (1) cellular mediated effects such as active anion secretion due to an activation of the adenyl cyclase and a consecutive increase if intracellular cAMP (Kimberg et al. 1971) or decrease of intestinal absorption caused by inhibition of Na,K-ATPase (Frizzell et al. 1976); (2) transepithelial filtration as a consequence of increased paracellular permeability (Rummel 1976).

Rat colon preparations absorb also in vitro sodium and chloride against a concentration gradient together with water (Parsons and Paterson 1965). Secretion, however, induced by secretagogues has never been observed under open circuit conditions in vitro. The lack of secretion in vitro was explained by assuming, that a driving force for secretion is located beyond the mucosa on its basolateral side, e.g., the hydrostatic tissue pressure in the subepithelial space physiologically caused by the capillary pressure. Theoretically it can be postulated that an increase of this subepithelial pressure could reverse the movement of water and water-soluble compounds in the opposite direction. Consequently, it has been shown, that secretagogues are able also in vitro to activate secretion if – for simulation of physiological conditions – an hydrostatic pressure was applied on the contraluminal side (Wanitschke et al. 1977b). It seemed possible – when testing in vitro different secretagogues all active in vivo – to discriminate between those secretagogues which decrease the paracellular resistance by increasing the epithelial permeability and those which activate secretion without increasing the epithelial permeability. This was the purpose of this study.

Send offprint requests to U. Karbach at the above address

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Methods

Experimental design. Sacs of stripped descending colon from Wistar rats (150–220 g) were mounted in a manner analogous to that of Parsons and Paterson (1965). The everted sacs were tied at one end to a glass tube and immersed in 50 ml electrolyte solution at 37° C. The sacs were filled through the glass tube. Hydrostatic pressure gradients were established by filling the sacs and the tubes to 2 cm and 7 cm H_2O above the fluid level of the beaker in which the sacs were immersed. The hydrostatic pressure gradients were maintained constant during the experiment by refilling or emptying the tubes in 15-min intervals to the initial fluid level.

Composition of solution. The mucosal and serosal solution had the following composition (in mmol/l): NaCl 109, KCl 4.5, NaH_2PO_4 0.2, Na_2HPO_4 1.8, $CaCl_2$ 2.5, $MgSO_4$ 2.5, $NaHCO_3$ 25, glucose 12.2. The pH value of the solution was 7.4 at 95% O_2/5% CO_2 saturation. The solution on the serosal side contained in addition either 5 μCi polyethylene-1,2-^{14}C-glycol 4000 (^{14}C-PEG) and 2 g/l meso-erythrol as volume marker and 10 μCi ^{14}C-erythrol and 5 g/l meso-erythrol as a marker to determine the paracellular permeability.

Water and electrolyte transport. Sodium and chloride concentration and ^{14}C-PEG activity were measured in the

serosal solution after 2 h incubation. Net water movement was calculated from the change in ^{14}C-PEG activity using standard formulas. Net sodium and chloride transport was calculated from the net water movement and the change of sodium respectively chloride concentration in the serosal solution. The sodium and chloride concentration of the transferred fluid was calculated from the net sodium and chloride transport and the net water movement. The electrolyte composition on the mucosal side did not change during the experiment because of the relative large volume of the mucosal solution (50 ml) when compared with the serosal volume of 1–2 ml. After extraction with diethyl ether the dry weight of the mucosa was determined. Transport rates are expressed per gram fat-free dry weight and 2 h incubation.

Paracellular permeability. Paracellular permeability was determined by the transfer of erythrol from the serosal to the mucosal solution. It has been shown that the transepithelial movement of erythrol (molecular radius 3.2 Å) provides a suitable method to assess changes of the paracellular permeability in the rat colon (Nell et al. 1977). After 2 h incubation ^{14}C-erythrol activity was measured in the mucosal solution. The amount of ^{14}C-erythrol which permeated the epithelium from the serosal to the mucosal side was calculated from the acitivity of ^{14}C-erythrol in the mucosal solution and the volume of the mucosal fluid. Movement rates of ^{14}C-erythrol were related to fat-free dry weight and 2 h incubation and are expressed as percentage of controls at 2 cm hydrostatic pressure.

Material and analysis. Deoxycholic acid ($3 \cdot 10^{-4}$ M), bisacodyl (10^{-5} M), ethacrynic acid ($2 \cdot 10^{-3}$ M) or rhein (6 mg%) were added to the mucosal solution. From literature it is known that these concentrations have the maximum effect to inhibit absorption in the rat colon in vitro (Wanitschke et al. 1977b, 1980; Wanitschke 1980). In a further experiment cholera toxin (25 µg/ml) was incubated in vivo in ligated loops of the colon for 6 h to bind the enterotoxin with the receptor (Hendrix 1975). In the ligated loop this concentration of cholera toxin effects fluid secretion (Goerg et al. 1980). The descending part of the pretreated colon was subsequently treated in vitro as described above but without cholera toxin in the bath solution.

Deoxycholic acid as its sodium salt was obtained from Merck AG, Darmstadt, FRG; rhein from Roth, Karlsruhe, FRG; cholera toxin by Wyeth Lab. Inc., Marietta, PA, USA; bisacodyl from Thomae, Biberach, FRG. Ethacrynic acid was kindly supplied by Sharp and Dohme, Munich, FRG. ^{14}C-PEG and ^{14}C-erythrol were obtained from New England Nuclear Chemicals, Dreieich, FRG; inactive PEG from Schuchardt, Munich, FRG; and meso-erythrol from Merck AG.

Sodium was determined by flame photometry and chloride by coulometrical titration. ^{14}C-activity was measured by a liquid scintillation counter (Packard Tricarb, model 3380).

^{14}C-activity in the rhein experiments was corrected by a quench curve obtained from different rhein concentrations.

Statistics. Results are given as the mean ± one standard error of the mean (SEM). Significances of differences were tested using the Student's *t*-test.

Results

Water and electrolyte transport

At a serosal hydrostatic pressure of 2 cm H_2O 8.4 ± 0.6 ml fluid per g d.w. and 2 h incubation was absorbed in controls (Fig. 1). The initial concentration of sodium (136 mmol/l) and chloride (116.5 mmol/l) in the serosal solution was increased to 154 ± 1 mmol/l sodium ($p < 0.001$) and 139 ± 1 mmol/l chloride ($p < 0.001$) after 2 h incubation under control conditions. The calculated sodium absorption was 2.3 ± 0.1 mmol and chloride absorption was 2.1 ± 0.1 mmol per g d.w. and 2 h incubation. The sodium concentration of the absorbate amounted to 291 ± 24 mmol/l and the chloride concentration to 282 ± 28 mmol/l when calculated from the net water transport and the movement of these ions. In other words the sodium and chloride concentration of the absorbate was more than twice as high as that in the bathing solution.

Net water movement was reduced to 30% (2.5 ± 1.2 ml per g d.w. and 2 h incubation, $p < 0.001$) of control value in sacs pretreated with cholera toxin. Similar to controls the final sodium concentration of the serosal fluid was increased to 152 ± 2 mmol/l and that of chloride to 133 ± 4 mmol/l. The calculated absorption of sodium (1.0 ± 0.2 mmol per g d.w. and 2 h incubation) and chloride (1.0 ± 0.2 mmol per g d.w. and 2 h incubation) was reduced by cholera toxin to 50% of control values ($p < 0.001$). The calculated sodium concentration of the absorbate was significantly increased to 406 ± 60 mmol/l

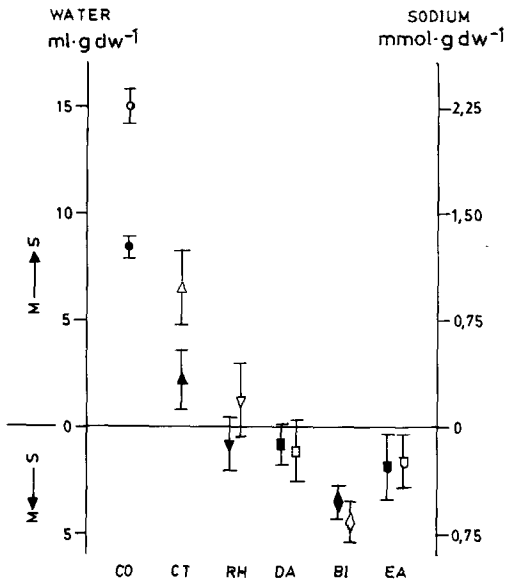

Fig. 1. Interference of different secretagogues on net sodium (*open symbols*, mmol per g d.w. and 2 h incubation) and water (*closed symbols*, ml per g d.w. and 2 h incubation) movement in stripped rat colon at a serosal hydrostatic pressure of 2 cm H_2O (mean values ± SEM; $n = 6–23$). Scales on the ordinate for water and sodium are chosen which makes it easy to recognize whether the fluid appearing on the mucosal or serosal side was unisotonic or isotonic. Isotonicity is indicated when the values for sodium and water were localized at the same point. Hypertonicity is indicated when the point for the value of sodium is significantly higher than that for water. (CO = controls, CT = pretreated with cholera toxin, RH = rhein, DA = deoxycholic acid, Bl = bisacodyl, EA = ethacrynic acid; concentration see Table 1; M = mucosa, S = serosa)

Table 1. Effect of hydrostatic pressure of 2 cm and 7 cm H_2O applied upon the serosal side on sodium and chloride concentration of the serosal solution in controls and under the influence of different secretagogues after 2 h incubation

Hydrostatic pressure	2 cm		7 cm	
C (mmol/l)	Na^+	Cl^-	Na^+	Cl^-
Initial solution	136	116,5	136	116,5
control	154±1	139±1	144±2	127±1
	$n=23$		$n=6$	
Deoxycholic acid	136±1	115±1	137±1	116±0,5
$3 \cdot 10^{-4}$ M	$n=8$		$n=8$	
Bisacodyl	135±1	115±2	135±1	113±1
10^{-5} M	$n=10$		$n=11$	
Ethacrynic acid	140±0,5	120±1	140±1	120±0,5
$2 \cdot 10^{-3}$ M	$n=7$		$n=7$	
Rhein	139±1	122±1	137±1	119±1
6 mg%	$n=8$		$n=12$	
Cholera toxin	152±2	133±4	146±1	127±0,5
25 µg/ml	$n=6$		$n=6$	

($p < 0.05$) and that of chloride even to 473±94 mmol/l ($p < 0.01$). Net water, sodium and chloride transport were abolished in the sacs exposed to rhein, deoxycholic acid and ethacrynic acid.

A higher hydrostatic pressure (7 cm H_2O) applied upon the contraluminal side of the epithelium affected the net movement of water, sodium and chloride much more pronounced (Fig. 2). In the controls net movement of water was completely abolished. The final concentration of sodium (144±2 mmol/l, $p < 0.05$) and chloride (127± 1 mmol/l, $p < 0.01$) at the serosal side was elevated above

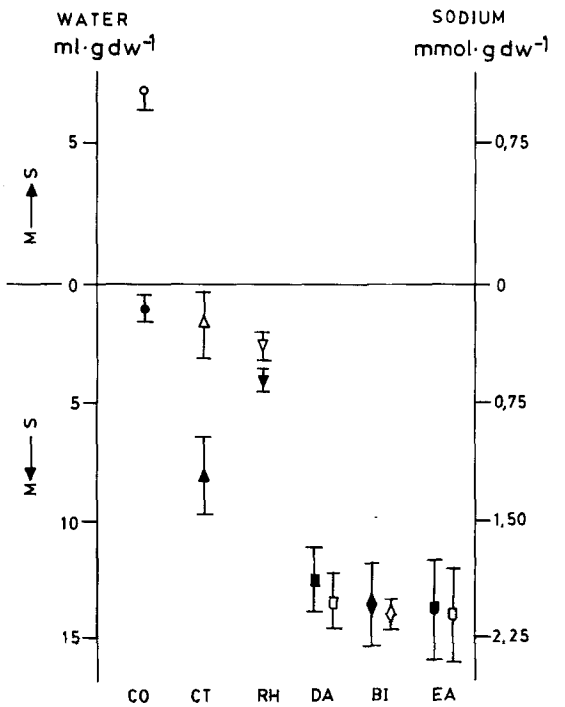

Fig. 2. Rate and direction of net sodium and water movement in the stripped rat colon and in presence of secretagogues at a serosal hydrostatic pressure gradient of 7 cm H_2O. Scale and symbols are identical with those in Fig. 1 ($n=6$–12)

the initial concentration but significantly lower when compared with the concentration of these ions in the serosal solution after 2 h incubation at the hydrostatic pressure of 2 cm H_2O ($p < 0.05$). At the hydrostatic pressure of 7 cm H_2O the calculated net movement of sodium (1.1± 0.1 mmol per g d.w. and 2 h incubation) and chloride (1.2±0.2 mmol per g d.w. and 2 h incubation) from the mucosal to the serosal side was diminished by 50% ($p < 0.001$). Under the influence of deoxycholic acid, bisacodyl and ethacrynic acid the same hydrostatic pressure caused an intense net water movement of about 13 ml per g d.w. and 2 h incubation ($p < 0.001$) from the serosal to the mucosal side together with sodium and chloride in isotonic proportion. Secretion of these ions in the presence of deoxycholic acid amounted to 2.0±0.5 mmol sodium and 1.9±0.2 mmol chloride per g d.w. and 2 h incubation. Bisacodyl induced net movement of 2.1±0.1 mmol sodium and 2.4±0.2 mmol chloride per g d.w. and 2 h incubation from the serosal to the mucosal compartment. Ethacrynic acid caused a secretion of 2.1±0.3 mmol sodium and 2.0±0.4 mmol chloride per g d.w. and 2 h incubation. In the presence of rhein the influence of 7 cm hydrostatic pressure on the net fluid movement was smaller. Only 3.2±1.5 ml fluid per g d.w. and 2 h incubation ($p < 0.05$) was transferred to the mucosal side. Secretion of sodium and chloride (0.5±0.1 and 0.2±0.2 mmol respectively per g d.w. and 2 h incubation) induced by rhein was significantly ($p < 0.01$) smaller than in the sacs exposed to deoxycholic acid, bisacodyl or ethacrynic acid. The sodium and chloride concentration of the serosal solution did not change when the sacs were incubated for 2 h with deoxycholic acid, bisacodyl, ethacrynic acid or rhein at 2 cm and 7 cm hydrostatic pressure.

After pretreatment of the epithelium with cholera toxin a volume of 8.1±1.8 ml fluid per g d.w. and 2 h incubation was secreted when applying a hydrostatic pressure of 7 cm H_2O ($p < 0.001$). The final sodium (146±1 mmol/l) and chloride (127±1 mmol/l) concentration were significantly increased ($p < 0.05$) when compared with the initial concentration of these ions in the serosal solution; they reached the same values as in the control group. Cholera

toxin induced only a small secretion of sodium (0.3 ± 0.2 mmol per g d.w. and 2 h incubation) and chloride (0.7 ± 0.2 mmol per g d.w. and 2 h incubation) at the serosal hydrostatic pressure of 7 cm H_2O. Consequently, in contrast to the groups of deoxycholic acid, bisacodyl, ethacrynic acid and rhein the fluid which appeared at the mucosal side of the epithelium pretreated with cholera toxin therefore was hypotonic. The calculated concentration of sodium in the secreted fluid was 52 ± 8 mmol/l and that of chloride 113 ± 39 mmol/l.

Paracellular permeability

In controls 12 ± 4% of the ^{14}C-erythrol applied at the serosal solution moved to the mucosal side at 2 cm hydrostatic pressure during 2 h incubation. The mucosal appearance of ^{14}C-erythrol in the controls was doubled when increasing the hydrostatic pressure to 7 cm H_2O ($p < 0.01$; Fig. 3). At 2 cm hydrostatic pressure the mucosal appearance of ^{14}C-erythrol under the influence of rhein did not differ from control values. But in contrast to controls the amount of ^{14}C-erythrol moved to the mucosal side was not enhanced by a 7 cm hydrostatic pressure in the epithelium treated with rhein.

In the tissue pretreated with cholera toxin the mucosal appearance of ^{14}C-erythrol was significantly diminished (40%; $p < 0.01$) and ^{14}C-erythrol movement was not influenced by the increased hydrostatic pressure.

On the other hand the permeability measured by ^{14}C-erythrol was remarkably increased under the influence of deoxycholic acid, bisacodyl, and ethacrynic acid. At a hydrostatic pressure of 2 cm H_2O the permeability was several times greater in the epithelia treated with these compounds than in controls. Deoxycholic acid caused a 6-fold, bisacodyl a 2-fold and ethacrynic acid a 6-fold increase ($p < 0.001$) of the ^{14}C-erythrol movement from the serosal to the mucosal side. At a hydrostatic pressure of 7 cm H_2O the amount of ^{14}C-erythrol which appeared at the mucosal side was under the influence of deoxycholic acid 3.5 times, of bisacodyl 2.5 and of ethacrynic acid 4 times that of controls ($p < 0.001$).

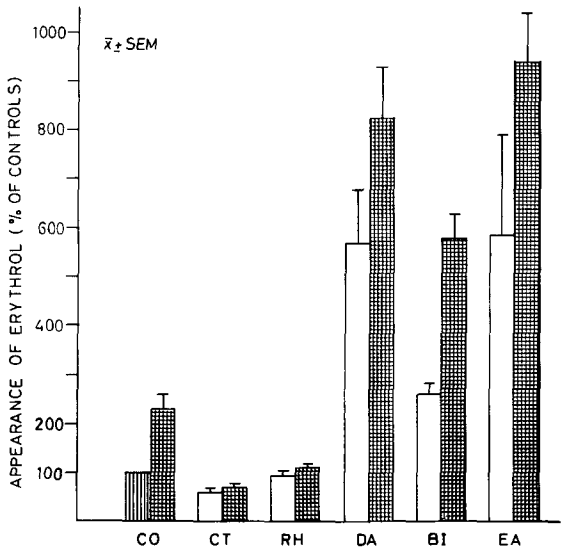

Fig. 3. Appearance of erythrol in the mucosal solution at a serosal hydrostatic pressure gradient of 2 cm H_2O (*white columns*) and 7 cm H_2O (*hatched columns*) and in presence of secretagogues. The values given are mean ± SEM of the ^{14}C-erythrol activity (cpm/g d.w. and 2 h incubation) in the mucosal solution in relation to controls at a pressure gradient of 2 cm H_2O (*striped column*), which was defined 100% and amounted to 92,279 ± 10,028 cpm/g d.w. and 2 h incubation

Discussion

In the colon sodium moves from the lumen against an electrochemical gradient to the serosal side by an active transport mechanism (Binder and Rawlins 1973). Accordingly to the model of Diamond and Bossert (1967) sodium is extruded from the cell by the Na,K-ATPase (Skou 1957) located at the basolateral membrane (Fujita et al. 1972) into the lateral intercellular space. Because of the relatively high sodium resistance by the tight junctions in the colonic mucosa a "standing osmotic gradient" is established. On the basis of the measured fluid transport and the sodium and chloride concentration in the serosal compartment it could be calculated that the tonicity of the fluid appearing on the basolateral side of the epithelium amounts 2–3 times that of the isotonic tyrode solution present at the luminal side when starting the incubation. Water movement from the cell or the lumen into the lateral space takes place in response to this osmotic pressure difference exerting a local hydrostatic pressure in the intercellular space. In vivo under normal conditions fluid than rapidly moves from this space into the capillaries.

In our in vitro experiments the subepithelial or intercellular pressure was modulated by a hydrostatic pressure applied upon the serosal side of the stripped rat colonic mucosa. In the control group at a 2 cm hydrostatic pressure a net movement of water, sodium and chloride from the mucosal to the serosal side occured. The absorption figures agree with other in vitro studies (Parsons and Paterson 1965; Wanitschke et al. 1977b). At 7 cm hydrostatic pressure, however, the net movement of water was abolished. Although net sodium and chloride transport was also decreased, these ions still were absorbed. The results are in good agreement with the findings of Wanitschke et al. (1977b), who have shown under the same experimental conditions, that net sodium and water transfer are linearly dependent on the serosal hydrostatic pressure gradient in the range of 3–20 cm. In their experiments net movement of water ceased at a pressure of 5.6 cm H_2O and of sodium at 11 cm H_2O. In the small intestine the reversal of fluid movement in the opposite direction by an increase of the hydrostatic pressure has been shown in several experiments in vivo and in vitro (for ref. see Lifson 1979). The hydraulic conductivity of the small intestinal mucosa is higher and in contrast to the results obtained in the colon no discrimination is found between sodium and water. The colon is capable to build up an osmotic gradient (Parsons and Paterson 1965), so that the final fecal water is hypotonic with respect to the plasma. This is in agreement with the different leakiness of these parts of the intestine (Fordtran et al. 1965). It is assumed that a net transport of fluid from the serosal to the mucosal side occurs when the capillary or the subepithelial pressure exceeds the hydrostatic pressure, which is established in the intercellular space by the sodium pump. The serosal hydrostatic pressure then induces streaming of fluid across the limiting junctions toward the luminal compartment (Rummel et al. 1975). In

vivo the subepithelial hydrostatic pressure, resulting from the oncotic and hydrostatic pressure along the capillary network, amounts 5–10 cm H_2O in the small intestine (Lifson 1979).

It is well established that cholera toxin induces net secretion of fluid and electrolytes by an active mechanism, e.g., by stimulating the adenylate cyclase (Kimberg et al. 1971). This is probably the reason for the inhibition of absorption in the colonic mucosa. Furthermore it has been demonstrated that cholera toxin reduces paracellular permeability of the colonic mucosa (Goerg et al. 1980). It is not known whether cholera toxin affects the tightness of the junctions (Duffey et al. 1981) or the decreased paracellular permeability is caused by a reduction of the lateral intercellular space of the colonic epithelium and by changes of the geometry due to the interdigitations, similarly to that described by DiBona et al. (1974) in the small intestine. As the sodium pump is not inhibited by cholera toxin, the functional asymmetry of the mucosa is maintained and can be influenced by the serosal hydrostatic pressure in the same way as in controls.

In the presence of the anthrachinone derivative rhein net fluid movement was abolished at 2 cm hydrostatic pressure. Since no sodium gradient was established in spite of a decreased paracellular permeability, the most plausible assumption is an inhibition of the sodium pump by the drug. This fits with the results of Wanitschke (1980), who has demonstrated that rhein in the same concentration as used in our experiments leads to an almost complete inhibition of the Na,K-ATPase in the rat colonic mucosa. In the presence of rhein the effect of the increased hydrostatic pressure is significantly less pronounced than in controls. This is probably the consequence of the swelling of the mucosa cells caused by an inflow of sodium and water into the cell after the inhibition of the Na,K-ATPase (Albus et al. 1979).

Under the influence of deoxycholic acid, bisacodyl, and ethacrynic acid net fluid absorption was abolished at 2 cm hydrostatic pressure. A pressure of 7 cm H_2O induced a remarkable movement of water, sodium and chloride in isotonic proportion from the serosal to the mucosal side. The permeability as indicated by ^{14}C-erythrol is increased pointing to an increased leakiness of the limiting junctions. When the resistance of the tight junctions is decreased, the efficiency of the pumping system is diminished or can be completely invalidated (Rummel 1976). Hence due to the increase of the paracellular permeability the serosal hydrostatic pressure acts as the dominant driving force for hydraulic filtration resulting in a passive net secretion of an isotonic fluid.

It has been shown that laxative acting bile acids increase intestinal permeability for a variety of water soluble compounds (Nell et al. 1975, 1976). The same effect has also been demonstrated for diphenolic laxatives (Nell et al. 1976, 1977). The permeability characteristics as well as the reversibility of the effect and careful histological controls (Goerg et al. 1980; Wanitschke et al. 1977a) confirm that the increase of the paracellular permeability caused by deoxycholic acid and diphenolic laxatives are due to an increased leakiness of the tight junctions without desintegration of the epithelium at least under the concentrations used in these experiments. Epitheliolysis of the colonic epithelium as described by Chadwick et al. (1976) were found at concentrations of dihydroxy bile acids ($6 \cdot 10^{-3}$ M) higher than used in our experiments ($3 \cdot 10^{-4}$ M).

There is some experimental evidence that bile salts (Binder et al. 1975) and diphenolic laxatives (Loeschke 1979) may act by increasing cAMP levels mediated by activation of the prostaglandin synthesis (Beubler and Juan 1978). These findings might be epiphenomena dependent on different concentrations of the substances. The results presented here favour the concept of secretion as hydraulic filtration due to an increased permeability as the predominant factor in the presence of deoxycholic acid and bisacodyl.

Wanitschke et al. (1980) have shown that ethacrynic acid given to the mucosal solution in a concentration of $5 \cdot 10^{-4}$ M inhibits water and sodium absorption in the isolated colon of the rat by 50%. The effects obtained with the same concentration of ethacrynic acid in our experiments are similar to that induced by deoxycholic acid and bisacodyl. These results evidence that secretion in the presence of ethacrynic acid is caused by hydraulic filtration as an consequence of an increased paracellular permeability. The treatment of the isolated gallbladder of the guinea-pig with 10^{-3} M ethacrynic acid applied to the serosal side results in epithelial cell damage (Petersen et al. 1979). In our experiments this concentration had no effect on fluid movement when given to the serosal side of the rat colonic tissue, whereas at the mucosal side a 75% inhibition of net water movement was demonstrable. It seems that rat colon is less sensitive to ethacrynic acid than the guinea-pig gallbladder. The increase of the paracellular permeability by loosening the junctions seems to be the dominating effect of ethacrynic acid in our preparation. An inhibition of the Na,K-ATPase, that has been shown in several epithelia treated with ethacrynic acid (Cassidy 1970; Duggan and Noll 1965; Heintze and Manjura 1975; van Os and Slegers 1970) could not be found in the rat colon (Wanitschke et al. 1980). Frederiksen (1978) did also not find an influence on Na,K-ATPase of ethacrynic acid given to the mucosal side of the rabbit gallbladder. The cAMP content of the rat colonic mucosa exposed to ethacrynic acid was also not increased (Wanitschke et al. 1980). Therefore we draw the conclusion from our results that in the rat colon ethacrynic acid acts primarily by decreasing the diffusional resistance of the paracellular pathway.

In conclusion, our results reinforce the view that the subepithelial hydrostatic pressure plays an important role in the intestinal water and electrolyte transport. Net fluid transport to the mucosal side in the rat colonic mucosa exposed to different secretagogues under in vitro conditions can be induced by an increase of the serosal hydrostatic pressure gradient. From the composition of the transferred fluid and the changes in paracellular permeability secretagogues acting on paracellular pathway can be distinguished from those with other mechanisms by the use of this simple in vitro test.

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