Cecal Enlargement Combined with Sodium Transport Stimulation in Rats Fed Polyethylene Glycol (36966)

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Within the intestinal tract of rodents, the cecum holds morphologically a special position in that it represents a pouch interposed into an otherwise cylindrical tube extending from the duodenum to the rectum. Little is known about cecal function except that the organ is a site of bacterial cellulose degradation which occurs to some extent even in monogastric animals like the rat (1). The cecum is reversibly enlarged under various conditions (2–5) including the germfree status (6–8). In germfree rats, the enlarged cecum was recently reported to absorb sodium and water in amounts severalfold above those measured in conventional controls even when the difference in mucosal surface area or wall dry weight was taken into account (9). It was suggested that the enlargement as well as the stimulation of sodium-coupled water transport may be connected with the presence of endogenous protein and carbohydrate compounds (10–12) which accumulate in the germfree cecum in the absence of bacterial degradation (10, 13) and elevate the intraluminal colloid osmotic pressure (14). To test this hypothesis, in the present experiments an undegradable, osmotically effective polymer was fed to normal rats to see whether they would also develop cecal sodium transport stimulation and growth. For comparison, similar measurements were carried out in the colon and ileum.

Methods. As a suitable molecule, polyethylene glycol 4000 (PEG, mol wt 3000–3700, Serva Chemicals, Heidelberg) was added to the drinking water of Wistar rats (both sexes, 150–250 g) in a concentration of 50 mmoles/kg. Due to the high osmotic coefficient of polyethylene glycol solutions (15), this fluid was isosmolal (300 mOsmol/kg) as measured by freezing point depression. Control rats were given tap water without PEG, both groups of animals having access to their drinking fluids and to standard rat chow (Altromin R, Altrogge, Lage) *ad libitum*.

Experiments were performed in inactin anesthesia (80 mg/kg intraperitoneally) while the animals were kept at 37° on a heated operating table. The *in vivo* sac technique to measure solute-coupled water flow has been described (9). In short, the cecum was ligated at both the ileal and colonic ends, the contents were removed through a small opening cut into the apex and replaced by a known volume of isotonic sodium chloride solution. With the sac placed back into the abdominal cavity, serial samples could be aspirated via thin polyethylene tubing leading into the lumen. Initial saline volumes were 3–5 ml in control and 3–20 ml in PEG rats. Since stretching influences sodium transport in some epithelial tissues (16) these volumes were aimed not to distend the cecal sac unduly at the beginning of an experiment but to leave a volume of at least 1.0 ml at its end. Under these circumstances, initial fluid volumes had no definite effect on absorption (Fig. 1). Measurements using ^{14}C-PEG (mol wt ca. 4000, New England Nuclear, Boston, Ma) as a nonabsorbable volume marker (recovery 95% in both animal groups) showed that absorption was linear with time over 3 hr (Fig. 2). Therefore, in the majority of experiments flow was determined volumetrically comparing the initial and final (3 hr) volume. Volume flow was referred to the gross surface area and to the dry weight of the excised sac, both parameters being determined as reported earlier (9).

In experiments utilizing the rest of the colon and the small intestine, the approach

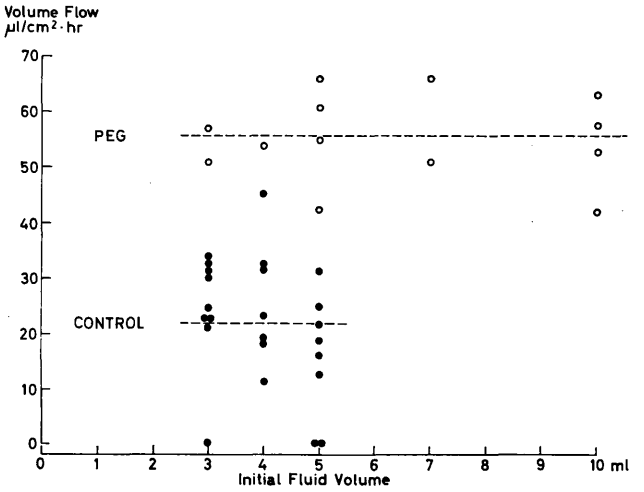

FIG. 1. Independence of solute-coupled water flow and initial fluid volume in cecal sacs. Values were derived from experiments in rats pretreated with polyethylene glycol 4000 for 7 days (○), and in controls (●). The difference between an initial volume of 3 and 5 ml in the control group is not significant ($p > 0.05$).

was analogous, *i.e.*, ligated sacs were formed of the entire colon (except the rectum) and of the ileum (15–20 cm upward from the ileocecal valve), respectively. After rinsing, a defined volume of saline (5 ml in the colon, 5–10 ml in the small intestine) was introduced into the sac and the remaining fluid was withdrawn after 2 hr.

Transmural electrical potential differences were measured using agar–Ringer's solution bridges connected to calomel electrodes. The luminal bridge was ligated into the cecal sac. The reference bridge was either in contact with the peritoneal surface or dipped into a beaker containing Ringer's solution in which the deskinned tail was also immersed. Both references gave identical results. Ringer's bridges were preferred to saturated KCl bridges in order to avoid potassium loss into the luminal fluid, and because the resulting tip diffusion potential was small and equal in control and PEG animals (see legend Fig. 4). Potentials were read on a Knick potentiometer and corrected for asymmetry potentials in the order of 1 mV.

Sodium and potassium concentrations (flame photometer), chloride concentrations (Marius chlor-o-counter), ^{14}C-analysis (liquid scintillation counter), osmolality (Knauer

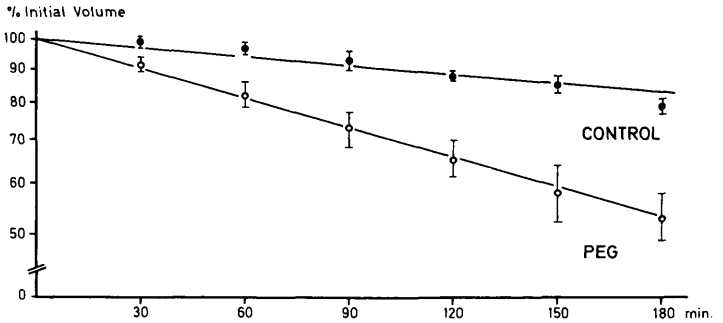

FIG. 2. Decrease of luminal fluid volume in cecal sacs as a function of time. The initial fluid volume was uniformly set at 100%. Means of 3 experiments/group in rats pretreated with polyethylene glycol 4000 for 7 days (○), and in controls (●), ± SEM.

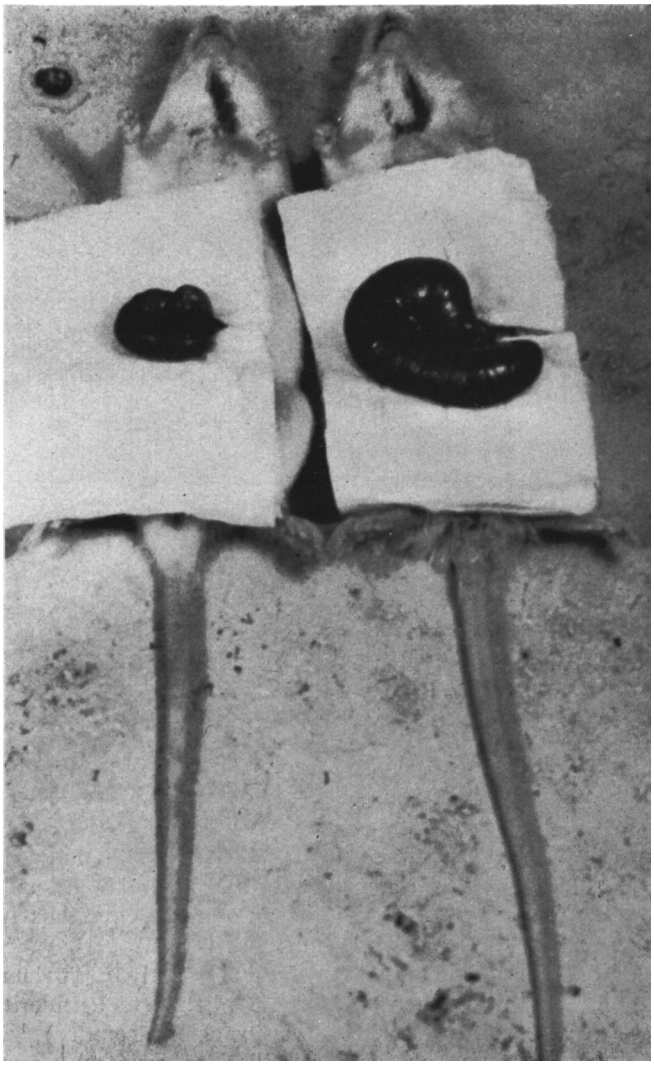

FIG. 3. Cecum of control rat (left) and rat fed polyethylene glycol 4000 for 2 mo.

osmometer) and blood hematocrit were measured using routine methods.

Results. Whole animal observations. PEG solution was readily drunk by the animals in amounts about twice the normal tap water intake. As in germfree rats, chronic diarrhea ensued, and the cecal contents became liquid. The PEG rats gained less weight than controls[1] although the daily uptake of rat chow was the same in both groups (16.5 ± 0.3 and 16.5 ± 0.4 g/day, respectively, $n = 25$ per group). This suggests a generally reduced nutritional status but PEG rats were also chronically dehydrated as indicated by an increase in serum osmolality, blood hematocrit, and serum sodium and chloride levels (Table I). Another observation was that PEG drinking induced polyuria, an aspect not further investigated.

Cecum enlargement. Figure 3 shows the degree of cecum enlargement seen after feeding PEG for 2 mo. From Table II, upper part, it can be deduced that the enlargement

[1] Total body weight, including the cecum, of PEG fed rats was approximately 5% less after 1 wk, 10% less after 4 wk and 30% less after 8 wk, although both groups had the same body weight initially.

TABLE I. Blood Values in PEG and Control Rats.

	Hematocrit (%)	Serum concn			
		Na (mEq/liter)	K (mEq/liter)	Cl (mEq/liter)	(mOsm/kg)
Control ($n = 10$)[a]	39.1 ± 0.9[b]	141.4 ± 1.1	4.8 ± 0.1	105.0 ± 0.4	294.2 ± 1.4
PEG[d] ($n = 10$)	42.2 ± 0.5[c]	146.2 ± 0.8[c]	4.5 ± 0.2	109.9 ± 0.9[c]	301.2 ± 2.3[c]

[a] n = no. of observations.

[b] SEM.

[c] $p < .025$ or better compared to control; the difference in potassium concentrations was not significant ($p > .1$).

[d] Animals on polyethylene glycol 4000 for 7 days.

was continuous during this time in terms of both surface area and dry weight, the former being finally tripled and the latter doubled. The discrepancy between increase in area and weight shows that the tissue did not simply grow as a whole but that it also became distended. A thinner cecal wall was also macroscopically evident, similar to observation in germfree rats (8, 17).

Solute-coupled water flow. In the absorption studies, isotonic saline solution was introduced into the cecum to measure the volume flow which is coupled to net sodium transport (18, 19). Table II, lower part, demonstrates that volume flow was already significantly enhanced after 2 days of PEG application. Maximum absorption per unit area was established within 7 days after which time only a slight further increase was found.

All further studies were carried out in 7 day animals. Table III lists the final electrolyte concentrations and the final osmolality of luminal fluid after 3 hr experiments. Sodium and chloride concentrations were statistically not different in PEG and control rats, but potassium concentrations and osmolality values were higher in the PEG group. When net sodium transport was calculated from volume flow and the initial (154 mEq/liter) and final sodium concentrations of luminal fluid, PEG rats were found to have absorbed sodium at twice the rate of controls, 10.0 versus 5.3 μEq/cm^2 hr.

The transmural electrical potentials recorded during solute-coupled water flow are presented in Fig. 4. Again a significant ($p < .001$) difference between treated and untreated rats was noted throughout the experiment, with larger luminal negativity in PEG rats. Direction and approximate mag-

TABLE II. Morphological and Functional Changes Induced in Rat Cecum by Polyethylene Glycol 4000.

Parameter	Control (Days): 0–56	PEG[a]				
		2	4	7	28	56
Morphological						
Surface area (cm^2)	15.7 ± 0.5[b]	16.1 ± 1.3	17.0 ± 1.4	21.5 ± 2.2[c]	35.8 ± 3.8[c]	50.9 ± 3.4[c]
Dry wt (mg)	149 ± 6	151 ± 13	142 ± 9	164 ± 11	231 ± 21[c]	284 ± 18[c]
Vol absorption						
μl/cm^2 hr	22.1 ± 2.3	40.5 ± 1.4[c]	50.3 ± 4.3[c]	55.3 ± 2.1[c]	57.4 ± 6.3[c]	58.1 ± 7.3[c]
μl/mg hr	2.1 ± 0.2	4.4 ± 0.3[c]	6.0 ± 0.5[c]	7.1 ± 0.4[c]	9.1 ± 1.3[c]	10.4 ± 1.4[c]
n[d]	24	5	5	13	7	7

[a] Animals on polyethylene glycol 4000 for the time indicated.

[b] SEM.

[c] $p < .005$ or better compared to control; other differences between PEG and control rats were not significant ($p > .05$).

[d] n = no. of observations.

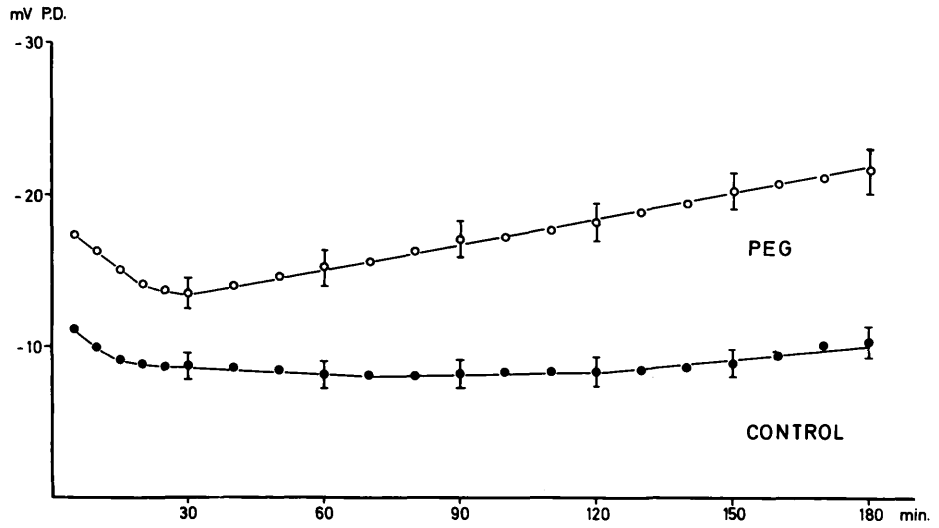

FIG. 4. Transmural electrical potential differences recorded in ceca of rats fed polyethylene glycol 4000 for 7 days (○), and of controls (●), during solute-coupled water flow. Values are uncorrected for small diffusion potentials (+ 1.5 – + 2.5 mV, identical in both groups) arising at the tip of the luminal Ringer's solution–agar bridge. Means of 8 experiments/group, ± SEM.

nitude of the potential are consistent with findings by others (20).

Reversibility. Three rats were kept on PEG solution for 4 wk, and then returned to tap water for another 4 wk. Both the cecum enlargement and the stimulation of solute-coupled water transport were no longer observed and thus were fully reversible.

Colon and small intestine. In germfree rats, the weight of the colon and small intestine differs only slightly from that of conventional controls (7), and water absorption studies in the small intestine did not reveal stimulation (21). It was therefore of interest to examine the effect of PEG feeding on the same parameters found altered in the cecum.

Table IV shows that feeding PEG for 7 days was without important effects in the colon and ileum. Most parameters measured were statistically not different in treated and control rats. This was true in all respects for the ileum. For the colon, an exception was the decrease in dry weight in PEG rats—probably a consequence of the less bulky content leading to muscular wasting. As a result of the diminished weight, volume absorption per unit weight was augmented whereas the absorption per unit area was unchanged. In 5 additional experiments of each group, the transmural electrical potentials were statistically not different in the colon of PEG and control animals.

TABLE III. Concentrations in Final Cecal Fluid.

	Control	PEG[a]
Na (mEq/liter)	126.7 ± 2.2[b]	124.1 ± 5.2
K (mEq/liter)	10.7 ± 0.6	23.5 ± 2.9[c]
Cl (mEq/liter)	65.8 ± 1.4	63.2 ± 4.2
(mOsm/kg)	268.4 ± 3.9	284.6 ± 3.4[c]

[a] Animals on polyethylene glycol 4000 for 7 days.
[b] SEM.
[c] $p < .01$ compared to control; other differences were not significant.

Discussion. Clearly, there is a far-reaching similarity in the observations made in ceca of germfree and PEG-fed conventional rats. Both groups of animals have chronic diarrhea and increased liquidity of cecal contents, both develop cecal growth and thinning of the cecal wall, and both absorb more sodium and water per unit cecal tissue. All these features are reversible within 4 wk in PEG rats and also in germfree rodents upon reestablishing their normal flora (22) except that in the latter the reversibility of sodium transport stimulation has not been studied. Even the chronic dehydration noted in PEG rats may

TABLE IV. Experiments in Colon and Ileum.

Parameter	Colon		Ileum	
	Control	PEG[a]	Control	PEG[a]
Morphological				
Length of sac (cm)	11.0 ± 0.3[b]	11.0 ± 0.3	19.0 ± 0.9	18.3 ± 1.1
Surface area (cm^2)	19.3 ± 0.7	19.5 ± 0.8	36.9 ± 5.3	37.9 ± 2.3
Dry wt (mg)	206 ± 5	156 ± 5[c]	349 ± 44	396 ± 40
Vol absorption				
$\mu l/cm^2$ hr	52.6 ± 6.1	57.3 ± 4.5	57.6 ± 6.4	56.9 ± 9.4
μl/mg hr	4.9 ± 0.6	7.3 ± 0.6[c]	6.0 ± 0.6	5.4 ± 0.8
Final luminal concn				
Na (mEq/liter)	114.4 ± 5.0	122.9 ± 3.1	144.0 ± 3.7	150.3 ± 2.0
K (mEq/liter)	15.5 ± 1.4	19.3 ± 1.1	8.0 ± 2.8	7.5 ± 0.3
(mOsm/kg)	233.6 ± 3.9	259.2 ± 4.5[c]	298.2 ± 5.3	309.0 ± 2.7
n[d]	10	10	5	6

[a] Animals on polyethylene glycol 4000 for 7 days.

[b] SEM.

[c] $p < .01$ compared to control; other differences between PEG and control rats were not significant ($p > .05$).

[d] n = no. of observations.

hold to a certain extent for germfree rats since higher values for red blood count, hemoglobin and percentage blood dry weight have been reported (7). Taken together, these findings suggest that the mechanism of cecal enlargement may well be the same in the two animal groups. Additional evidence that cecal enlargement is not unique to the bacteria-free status comes from the fact that it can also be produced in normal rats by feeding a variety of other substances such as kaolin (3), a number of poorly digestible polysaccharides (2), and antibiotics (4, 5).

A common factor in all of these conditions may be the accumulation of some large molecules within the cecal lumen. In germfree and, by analogy, in antibiotic-treated rodents, these seem to be mucoid substances originating from the gastrointestinal tract (10–12) and their role in cecal enlargement has been suggested earlier (10, 23). If osmotically effective, these molecules must increase luminal water content as was actually described in most of the above situations. Via elevated intraluminal pressure, the wall distension seen in germfree and PEG-fed rats could then be understood. If distension played any part in the additional changes observed, it might also be plausible that the colon and ileum did not show sodium transport stimulation and growth in PEG-treated or germfree (7, 21) rats. Both organs are morphologically open tubes as opposed to the sac-like structure of the cecum, and would neither accumulate material nor did they become enlarged.

However, why the distended cecum should also increase its (dry) weight and transport activity, is presently not apparent. In both germfree (9) and PEG-treated rats, solute coupled water transport per unit area or weight was stimulated drastically. In PEG rats, additional measurements showed higher luminal potassium concentrations and osmolality during solute-coupled water flow, as well as larger electrical negativity of the lumen. Since the transmural electrical potential appears to result, under these conditions, mainly from active sodium pumping (24), this finding supports the notion that the latter was stimulated in the cecum of rats given PEG as previously suggested for the germfree cecum (9). Such an interpretation is also compatible with preliminary measurements in PEG-fed rats demonstrating lower intraluminal sodium concentration yet larger electrical negativity in the stationary state (25) and higher Na^+-K^+-ATPase activity

of cecal mucosal homogenates compared to controls (unpublished data).

Summary. The organ enlargement and stimulation of solute-coupled water transport observed in the cecum of germfree rats can also be produced in conventional rats by adding polyethylene glycol 4000 to their drinking water. In these rats, the electrical potential difference across the cecal wall is increased during solute-coupled water flow. The structural and functional changes induced by polyethylene glycol are reversible and do not extend to the rest of the small intestine and the distal ileum.

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