Threshold Levels of NaCl Upholding Solute-coupled Water Transport in the Cecum of Germfree and Conventional Rats[1] (37236)

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(Introduced by T. Z. Csáky)

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It has been suggested that certain characteristics of germfree rodents, namely, cecal enlargement, semi-liquid contents of the lower bowel, and constant mild diarrhea (1) develop on the basis of abnormal composition of intestinal contents and on consecutive water-absorption inhibition. Thus, in cecal contents of germfree rats, the following have been observed in comparison to conventional controls: (a) marked concentration of muco-proteins (2–4), and (b) very low levels of bicarbonate and chloride ions (5–7). (c) The levels of cations, referring, mainly to sodium and potassium, were found unchanged or only slightly reduced (5–7). On feeding chloride-yielding resin to germfree rats, considerable improvement of water absorption could be obtained (8). In addition, (d) total osmotic pressure was found to be isotonic (9) and (e) colloid osmotic pressure pronouncedly hypertonic (10) in comparison to these values in blood plasma.

It is well known that in conditions of isotonicity on either side of the intestinal membrane, solute transfer and water transfer are closely linked (11–14). Under such a condition, the absorption of water appears to be secondary to the inward pumping of solutes, mainly of sodium chloride. Below a certain critical level of sodium ion in the gut contents, this form of water absorption comes to a stop. Thus, it appears that the excess of macromolecular compounds in the germfree lower gut, originating from secretions and from mucosal desquamation, is responsible for binding cations and replacing diffusible anions, which in turn "starves" the mucosa in the process of solute coupled water absorption. In conventional animals, the intestinal microflora seems to be responsible for the degradation of these colloids. It was found that the lower bowel of germfree rats lacks certain mucinases, while such enzymes are present in intestinal contents of conventional controls (15).

In reference to the ileum of germfree rats, on replacing the natural contents with saline in a short-term, perfusion-type experiment, an initial lag was observed in water absorption (16). This difference from conventional controls tended to disappear with the progress of the experiment. Repeating this work in ligated, saline-filled ceca of rats, water absorption from germfree specimens was found greatly elevated in comparison to that of conventional controls (17). This indicated that, in the presence of an adequate supply of ions needed for the maintenance of solute-coupled water absorption, the germfree intestinal mucosa is fully capable of performing normally in this respect. However, if instead of saline, the supernatant of germfree cecal contents was introduced into the ligated cecum, then no absorption of water took place. Indeed, under these circumstances, a slight efflux of water from tissue to lumen occurred (7).

In order to further investigate the improved water absorption from the germfree cecum when it is relieved from its natural contents, this work aimed (a) to study the time course of Na^+ and water transport from the saline-filled cecum and (b) to determine the threshold levels (steady-state con-

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centrations) of NaCl in the cecal lumen of germfree and conventional rats, which are needed for maintaining sodium coupled water absorption.

Methods and Materials. Experimental animals were germfree and conventional Fischer 344 (CDF, Charles River) 10–12-month-old male rats weighing 300–400 g. The diet was steam-sterilized L-462 (18). The germfree rats were housed in flexible plastic isolators (19); the conventional controls were kept in the same air-conditioned room in the open enviroment. Individual housing was in rigid plastic cages on corn-cob-type bedding, 2–3 rats/ft^2 floor space. Before the day of the experiment, the rats were fasted overnight. All observations were carried out in the open laboratory environment. Through an abdominal midline incision in urethane–anesthesized rats (150 mg/100 g body weight), the ileum and colon were ligated at their emergence from the cecum, leaving intact the circulation of this area. Through an opening cut at the apex of the cecum, its contents were removed and the lumen repeatedly rinsed with lukewarm isotonic mannitol solution.

(a) *Time-course study of absorption.* In this experiment, a cannula tied to the apical cut was connected to an infusion pump, thus permitting mixing and withdrawal of samples from the cecal perfusate. The solution was 0.9% NaCl, containing labeled PEG (Polyethylene-1,2-^{14}C glycol, mol wt ca. 4000, New England Nuclear Corp., Boston, MA). In the case of germfree rats, the volume of the perfusate was 25 ml; in the case of the conventional controls, it was 10 ml. The rate of perfusion was 12.4 ml/min. The experiment lasted for 5 hr, and samples of the perfusate were taken at hourly intervals.

(b) *Determination of NaCl concentration needed for maintaining water absorption.* Graded solutions of NaCl and mannitol (ranging from 0 to 155 m*M* NaCl with mannitol added to reach isotonicity with blood, *i.e.*, 300 mOsm) containing labeled PEG were introduced into the ligated cecal pouch which was then replaced into the peritoneal cavity. In the case of germfree rats, the liquid input was 15 ml; in the case of the conventional control, it was 6 ml. During the 5-hr duration of the experiment, the pouch contents were quantitatively removed at hourly intervals and used for analysis. After this, the pouch was rinsed with isotonic mannitol, and a new test solution was introduced. In general, the experiment was started by using lower concentrations of NaCl, the higher ones following thereafter. Occasionally, the sequence of the solutions used was random. Either approach gave essentially similar results within comparable groups. This mode of scheduling the experiment was made necessary by the limited availability of germfree rats.

(c) *Terminal procedures.* The cecal contents were quantitatively collected, their volume was taken, and PEG concentrations were determined in a scintillation counter before and after the experiment (Tri Carb, Packard). From these data, net water absorption was calculated. The recovery of PEG was always above 95%. In the solutions used (terminal samples were spun in a clinical centrifuge), the following were determined: osmolality (osmometer, Advanced Instruments, Newton, MA), Na^+ (Flame photometer, Baird Atomic, Hinsdale, IL) and Cl^- (Chloridometer, Buchler-Cotlove, Fort Lee, MI). From the latter, the net transport of these ions was calculated. The surface area

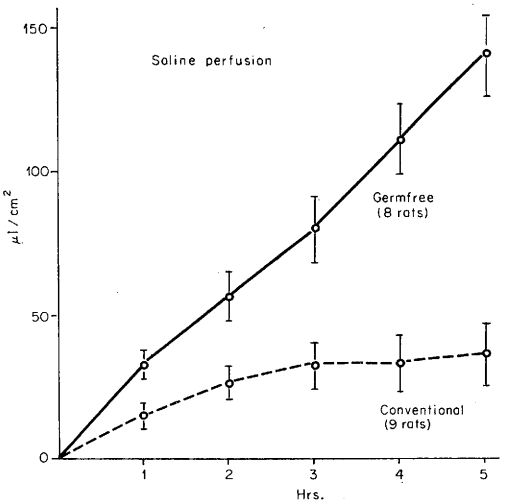

FIG. 1. Time course of water absorption from cecal pouches; arithmetic means and standard deviations are given.

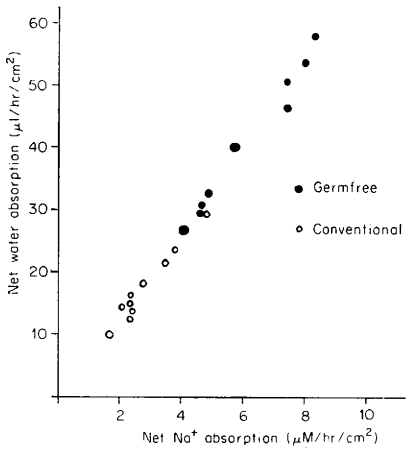

FIG. 2. Relationship between net Na^+ and net water absorption from cecal pouches.

of the cecal mucosa was determined by a previously described procedure (17). In these studies, 16 germfree and 18 conventional rats were used.

Results. Figure 1, showing the time course of water absorption from cecal pouches, indicates substantially higher values for the germfree groups in comparison to conventional controls. After an approximately 3-hr run, the latter reached a plateau, while in the former, at the end of the 5th hour, water absorption was still in progress. Figure 2 illustrates that net absorption of Na^+ and water were closely linked in both groups of animals and showed a similar quantitative relationship.

Figure 3 and Table I illustrate the threshold levels of NaCl in luminal fluid which are needed for maintaining solute-coupled water absorption from cecal pouches. In case of germfree rats, this threshold, *i.e.*, the level of NaCl at which net water absorption falls to zero, was reached between 15 and 0 m*M*, while in case of conventional controls it was between 77 and 40 m*M*, respectively. It is also shown in Table I that in the case when saline was used as luminal fluid (at 155 m*M* NaCl), net absorption of water, Na^+, and Cl^- per time and surface unit showed approximately double values in the germfree cecum in comparison to its conventional counterpart. The parallelism between net absorption of water, Na^+, and Cl^-, which was partly indicated in the time-course experiment, was evident in the present instance.

Discussion. This work clearly indicates that, on replacing the natural contents of the cecum in germfree rats with saline, this organ, in terms of solute coupled water absorption, will outperform conventional controls. Thus, in the latter group, water absorption will come to a stop at a transmural gradient of 70 m*M* Na^+, while in the former group, with a gradient of well over 100 m*M* Na^+, water absorption is still active (calculated from Na^+ values in blood plasma and cecal lumen). The greater efficiency in water absorption of the germfree cecal pouch, when filled with saline, was also indicated by the

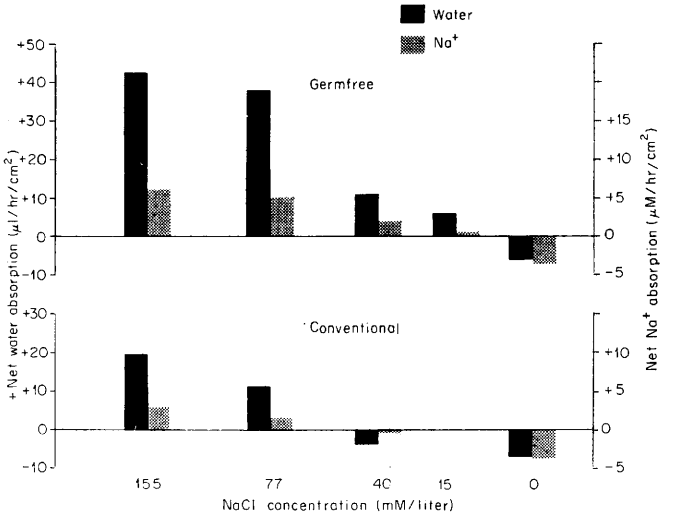

FIG. 3. Luminal concentration of NaCl and net absorption of water and Na^+ from cecal pouches (negative numbers indicate efflux from tissue of lumen).

TABLE I. Net Absorption of Water, Na^+ and Cl^- from Ligated Cecal Pouches Holding Solutions of NaCl in Graded Concentration.

Group	Initial NaCl concentration in cecal solution (m*M*)[a]	Absorption/hr/cm² mucosal surface area		
		Water (μl)	Na^+ (μM)	Cl^- (μM)
Germfree (8 rats)	0	−5.66 ± 2.54[b]	−3.59 ± 0.44	−0.70 ± 0.12
	15	6.49 ± 1.19	0.27 ± 0.12	2.01 ± 0.26
	40	11.03 ± 1.65	1.80 ± 0.45	4.51 ± 0.88
	77	37.63 ± 4.67	5.60 ± 0.55	9.50 ± 1.46
	155	42.62 ± 5.49	6.21 ± 0.77	12.24 ± 2.19
Conventional (9 rats)	0	−7.55 ± 0.93	−3.35 ± 0.24	−0.90 ± 0.11
	40	−2.69 ± 1.24	−0.02 ± 0.09	2.94 ± 0.54
	77	10.40 ± 2.58	1.40 ± 0.51	5.13 ± 0.61
	155	19.04 ± 2.69	2.78 ± 0.66	6.92 ± 0.71

[a] All cecal solutions were adjusted to 300 mOsm by adding mannitol, when applicable.

[b] Negative numbers indicate efflux from tissue to lumen. Arithmetic means and standard deviations given.

time-course experiment. This confirms previous observations, though in the present instance the ratio between germfree and conventional values (in reference to the first 3 hr of the experiment) was 2.3, while in previous work values over 5 (17) and 3–4 (7), respectively, were found. Differences in the techniques adopted (the present work used infusion and withdrawal of luminal fluid; the former did not), might be responsible for this variation. On the other hand, the relative efficiency of Na^+-coupled water absorption of the cecal mucosa (amount of water absorbed per unit Na^+) remained essentially the same (around 600 μl water/100 mmole Na^+ absorbed) both in the germfree and conventional groups, irrespective of whether the time course or threshold experiments were conducted (except when in the latter very low levels of Na^+ were used). The net transport of Cl^-, by and large, paralleled that of Na^+. Thus, the present findings, along with others (7, 17), emphatically support the thesis that there is no inherent defect in the germfree cecal mucosa which might explain inhibited water absorption from the lower bowel of these animals. The problem, as suggested by other work (6, 7), appears to be rather in the accumulation of mucus and in ionic shifts of its contents.

The mechanism of these characteristics of the germfree cecal mucosa remain unknown. It is possible that morphologic features portraying greater surface area contribute to it. Recently it was found (20) that in the cecal mucosa "epithelial cells of germfree rats were longer and more slender" and that "the number of cells supported by a given area of basement membrane was higher in germfree animals than in conventional animals." It was also found in this work that the microvilli of these cells were on the average 25% longer than in conventional controls.

It is possible that the presently described greater efficiency of the cecum in germfree rodents represents a compensatory mechanism to the constant state of water-absorption inhibition which, induced by microbially-undegraded, abnormal intestinal contents, characterizes this organ.

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