PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 541–545 (1975)

Electrical Resistance and ATPase Levels in the Cecal Wall of Germ Free and Conventional Rats[1] (39074)

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(Introduced by T. Z. Csaky)

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Previous studies have indicated that in the lower bowel lumen of germ-free rodents, the accumulation of mucus and of related substances (1–3) results in elevated levels of colloid osmotic pressure (4) and in trapping of cations which are needed for the maintenance of solute coupled water absorption (5). These conditions appear to be responsible, among others, for reduced Na^+ and water absorption, for the more liquid contents of the lower bowel and for the ensueing chronic mild diarrhea that characterizes germ-free rodents (5, 6). A similar form of water absorption inhibition could be produced also in conventional rats whose diet was supplemented with the nonabsorbable polymer polyethylene glycol (7). In the cecal mucosa of these PGG-fed animals elevated levels of Na^+ and K^+ stimulated ATPase activity could be shown (8).

The present work aimed to answer the following questions: (a) Do the water and electrolyte anomalies observed in the germfree cecum modify its transmural electrical resistance? (b) In affirmative case, can the wall's electrical resistance be modified when instead of physiological solution, the supernatant of germfree cecal contents is bathing the cecal mucosa? (c) What are the levels of total and Na^+ and K^+ stimulated ATPase activity in mucosal samples obtained from the cecum of germfree and conventional rats?

Materials and methods. The experiments were performed in germ-free (GF) and conventional (CV) Sprague–Dawley male rats weighing 240–260 g, 2 months old, fed sterilized Ch.R. diet. These animals were sent by the breeder (Charles River Breeding Labs, Wilmington, Mass.) in GF and CV shipping isolators well supplied with food and water, by air express from Boston, Mass., to Milan, Italy. The time lapse between the rat's shipping date and their sacrifice at the laboratory in Milan did not exceed 2 days. On arrival, the rats were removed from the isolators, placed under pentobarbital sodium anesthesia (100 mg/kg body weight, ip) and killed by exsanguination in the open laboratory environment. The terminal procedures did not take more than 20 min, counting from the animal's removal from the isolators. Sterility of the germ-free rats was ascertained following routine procedures (9) by taking bacterial cultures from aseptically drawn samples of cecal contents.

Measurements of electric resistance across the cecal wall. The cecum, opened lengthwise and thoroughly washed with saline, was held between two lucite chambers (t in Fig. 1). The exposed area of the cecal wall was 0.63 cm^2. The electrical resistance was measured by passing dc pulses (100$\mu A/cm^2$/sec) through the tissue, using Ag/AgCl electrodes and recording the change in potential between two agar–KCl bridges (AA and BB, respectively, in Fig. 1). A correction to estimate the actual potential drop across the wall was obtained by measuring the potential differences between the BB bridges, without the tissue, either in Krebs–Henseleit bicarbonate solution or in the supernatant fluid ("c" and "d" in Fig. 1). On the basis of these determinations the transmural resistance (R_t) was calculated. Three types of resistance measurements were carried out, with the tissue bathed on the luminal side by Krebs–Henseleit bi-

[1] This work was supported by the State University of Milan and by U.S. Public Health Service (Grant No. AM-14621).

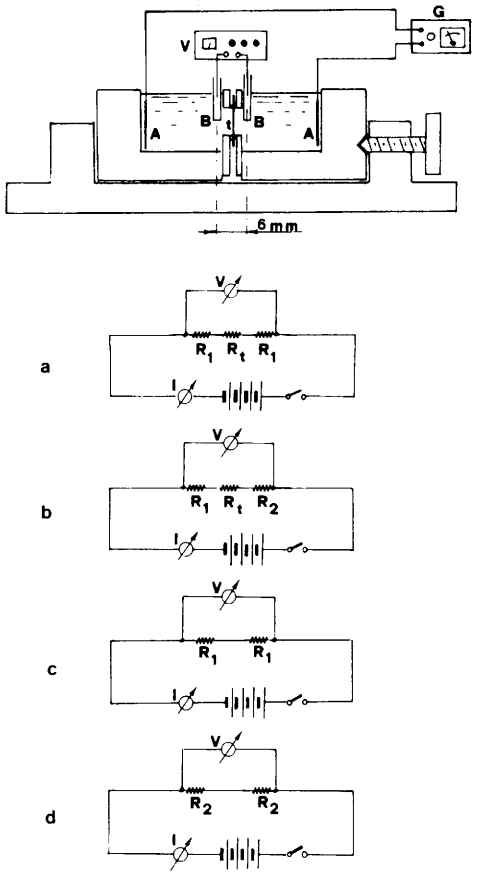

FIG. 1. Apparatus assembly for the measurement of the ohmic resistance (R_t) through the tissue (t) and experimental schemata (a, b, c, d); R_1, resistance of the Krebs–Henseleit bicarbonate solution; R_2, resistance of the supernatant fluid from the cecal content; AA, Ag/AgCl electrodes; BB, agar–KCl 3 *M* bridges; V, voltmeter for measuring the potential drop between the bridges; I, microamperometer; G, dc pulse generator.

carbonate solution (Steps A and C in Table I and a in Fig. 1) or by the supernatant fluid, obtained by centrifugation of cecal contents (8000*g*, 10 min) from GF rats (Step B in Table I and b in Fig. 1). The serosal side of the tissue was bathed in each case by Krebs–Henseleit bicarbonate solution. The resistance measurements were repeated until the constant values reported in Table I was obtained. Each experimental period lasted 10–15 min.

Preparation of mucosal homogenates. The ileum, cecum, and colon were removed and immediately washed and irrigated with cold 0.32 *M*-sucrose, 2.5 m*M*-EDTA adjusted to pH 7.5 with Tris OH. All further operations were performed at 4°. The intestinal segments were everted, and the mucosa removed by scraping with a glass slide following an established method (10). The mucosal scrapings were then weighed, placed each in 9 vol of 5 m*M* EDTA adjusted to pH 7.5 with Tris–OH and homogenized in a Sorvall Omni-Mixer for 60 sec. The homogenates were filtered through No. 25 bolting silk.

Enzyme determinations. ATPase activity was measured (11) under two conditions (a) in the presence of Mg^{2+}, Na^+, and K^+, and (b) in the presence of the same ions with ouabain added. The first measurements were taken to portray "total ATPase," the second ones to show "ouabain-insensitive ATPase." The "Na^+ and K^+ stimulated ATPase" was calculated by subtracting the ouabain-insensitive ATPase values from those of the total ATPase. The assay system of total ATPase contained 50 m*M* Tris–HCl, 7.4 m*M* $MgCl_2$, 20 m*M* KCl, 110 m*M* NaCl, 5 m*M* ATP, Na_2, homogenate 200 μl (pH 7.5). The system for the measurement of the ouabain-insensitive ATPase had the same composition with 1 m*M* ouabain added. The total volume of the reaction mixture was 2.5 ml and the temperature was 37°. The reaction was started by adding ATP to the systems. Samples were taken at 0, 5, and 10 min and the reaction was terminated by the addition of 50% trichloroacetic acid (w/v). P_i released was determined in double measurements by two separate methods (12, 13). The protein content of the samples was estimated by biuret method (14).

Results. The data reported in Table I show that the transmural resistance of GF rats is higher than in the CV control rats, when the luminal fluid is a Krebs-Henseleit bicarbonate solution (Steps A and C) and that the transmural resistance increases in both CV and GF rats, when the supernatant from the cecal content of GF rats is used as a mucosal fluid (Step B). Such an increase is significantly greater in the GF than in the CV rats (Step B). The elevated levels of transmural resistance recorded in case of GF

TABLE I. ELECTRICAL RESISTANCE (ohm-cm²) of CECAL WALL ORIGINATING FROM GERM-FREE AND CONVENTIONAL RATS; EFFECTS OF GERM-FREE RAT CECAL SUPERNATANT ON RESISTANCE ON MUCOSAL APPLICATION.[a]

Number of Rat	Step A Mu: Krebs–Henseleit solution Se: same	Step B Mu: GF cecal sup. Se: Krebs–Henseleit solution	Step C Mu: Krebs–Henseleit solution Se: same	Difference between Steps A and B
		Germ-free tissue		
1	90.0	144.0	81.0	54.0
2	113.4	138.6	113.4	25.2
3	102.0	147.6	84.6	45.6
4	126.0	157.5	126.0	30.9
5	88.2	126.0	88.2	37.8
6	124.0	154.3	138.6	28.3
7	141.7	185.8	135.4	44.1
8	144.9	192.1	151.2	47.2
Mean ± SEM	116.5	155.7	114.8	40.4
	7.7	8.0	9.1	3.3
		Conventional tissue		
1	96.1	112.6	96.1	16.5
2	94.5	119.7	113.4	25.2
3	91.3	124.4	91.3	33.1
4	99.2	122.1	92.9	22.9
5	91.3	130.7	91.3	39.4
6	97.6	111.8	97.6	14.2
Mean ± SEM	95.0	120.2	97.7	25.2
	1.3	2.9	3.4	3.9

[a] M = arithmetic mean. SEM = standard error of the mean. Mu = mucosal side. Se = serosal side.

rats and of those CV rats whose mucosa was exposed to GF cecal supernatant, might be related to a decrease in the mean ionic concentration in the epithelial tight junctions and/or to a decrease in the width of the intercellular channels. Such effects might be due to diminished transepithelial transport of Na salts (mucosa–serosa) and to the opposite osmotic flux (serosa–mucosa), created by the nonabsorbable molecules of the cecal content (5). The increase in transmural resistance, however, is greater in the GF than in the GF supernatant exposed CV rats, which suggests that differences in the properties of the two types of epithelial linings are likely to exist. This has recently been shown by morphologic observations in the cecal mucosa of GF and CV rats (15).

Table II indicates that the activity of both total and Na^+ and K^+ stimulated ATPase was elevated in all GF specimens in comparison to CV controls. This elevation, particularly in reference to Na^+ and K^+ stimulated ATPase, was marked in case of the ileum and cecum and approached or reached the levels of significance between our two groups of animals. The elevated levels of Na^+ and K^+ stimulated ATPase found in the mucosa of the GF cecum may be associated with the greater "absorptive efficiency" which this tissue displays when it is relieved from luminal inhibitory substances. Thus, in ligated cecal pouches of GF rats *in vivo* whose natural contents were replaced with saline, substantially higher values of Na^+ and water absorption have been found per unit surface than in CV controls (5, 16). In another study conducted in similar preparations, the threshold levels of Na^+ which are needed for the maintenance of active Na^+ and water transport were found markedly lower in GF specimens in comparison to CV controls (17). The greater absorptive efficiency of the germfree cecal mucosa might be considered as an adaptative change which this tissue musters

TABLE II. ATPase in Mucosal Homogenates Prepared from the Intestine of Germ-free and Conventional Rats.[a]

		Number of rats	Protein, total mg/g wet mucosa	Total ATPase		ATPase (Na^+-K^+) stim.	
				μmole P/min/g wet mucosa	μmole P/min/mg total protein	μmole P/min/g wet mucosa	μmole P/min/mg total protein
ILEUM	GF	12	98 ± 5	27.1 ± 1.4	.298 ± .019	9.3 ± 1.2	.104 ± .017
	CV	8	92 ± 5	23.7 ± 1.7	.265 ± .015	5.6 ± .9	.063 ± .010
	P*		>0.4 (107)	>0.1 (117)	>0.2 (112)	<0.05 (166)	<0.1 (165)
CECUM	GF	11	77 ± 7	20.5 ± 1.5	.287 ± .021	2.7 ± .5	.038 ± .008
	CV	10	75 ± 4	14.0 ± 0.7	.190 ± .010	1.6 ± .3	.021 ± .004
	P		>0.7 (103)	<0.01 (112)	<0.001 (152)	<0.1 (168)	<0.1 (181)
COLON	GF	11	83 ± 9	25.3 ± 1.4	.318 ± .028	7.8 ± 1.0	.107 ± .022
	CV	7	79 ± 4	19.5 ± 1.8	.262 ± .028	6.7 ± 1.1	.089 ± .015
	P		>0.7 (105)	<0.05 (130)	>0.1 (121)	>0.4 (116)	>0.5 (120)

[a] Arithmetic means and standard errors of the mean are given.
* P = statistic p and in parenthesis, % GF values assuming CV values = 100%.

in response to the state of chronic absorption inhibition.

Open problems are still the mechanism of the observed changes in ATPase level and the identification of the substances responsible for this change. The elevated ATPase levels in the GF gut mucosa could be due to an effect on the rate of formation or degradation of the enzyme protein, or to a change in the state of activity of the enzyme molecule. The substance (or substances) responsible for such effects might be bioactive metabolites in intestinal contents which in CV animals are degraded by the flora and which in GF counterparts accumulate and are available for absorption. Effects of this type have been observed in the case of steroid metabolism (18). Furthermore, some attention must be paid to the fact that the life span of intestinal epithelial cells is increased in GF rats compared to conventional rats (19).

Summary. (a) Electrical resistance across the cecal wall of germfree rats is higher than in conventional controls when the luminal fluid is a physiological solution. Transmural resistance of the cecum increases both in germfree and conventional rats when the supernatant from cecal contents of germfree rats is used as luminal fluid. These phenomena might be due to an effect of germfree intestinal contents on the ionic composition and/or on the dimensions of intercellular pathways within the intestinal epithelium. (b) Total and Na^+ and K^+ stimulated ATPase show higher levels in mucosal scrapings from the ileum and cecum of germfree rats than in conventional controls. This characteristic of the germ-free lower bowel may be associated with the greater "absorptive efficiency" which it was found to display when relieved from luminal inhibitory substances.

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June 26, 1975, P.S.E.B.M. 1975, Vol. 150.