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Elevated Levels of Colloid Osmotic Pressure in Cecal Contents of Germfree Animals[1] (38740)

H. A. GORDON AND SACHIKO NAKAMURA[2]
(Introduced by T. Z. Csaky)

Department of Pharmacology, College of Medicine, University of Kentucky, Lexington, Kentucky 40506

The present work was undertaken to observe whether osmosis is involved in maintaining in germfree rodents semiliquid the contents of the lower bowel resulting in mild diarrhea, which was first observed in guinea pigs by Nuttal and Thierfelder at the turn of the century (1). In absence of the intestinal microflora it appears that in conjunction with increased hydration, mucus and other macromolecular substances accumulate in the intestinal contents of these animals. Thus in cecal contents and feces of germfree rats elevated levels of mucopolysaccharides and of their components have been found in comparison to conventional controls (2–5). In terms of related enzymes, it was observed that in the feces of germfree rats, activity destroying mucopolysaccharide-type antigen (ABH(O) blood group antigen) was absent, whereas it was present in conventional rats (6). Recently it was found that in addition to mucus-like substances, undegraded dietary components may also contribute to the "macromolecular pool" of the germfree intestine (7). In reference to total osmolality, intestinal contents of germfree rats have been found essentially isosmotic with blood, whereas they were hypertonic in conventional controls (8).

Mucus-like substances adhering to the intestinal wall in germfree rats appear to inhibit water absorption from the ileum of these animals (9). In a preliminary note we have indicated that colloid water attraction exerted by these compounds in intestinal contents, might be implicated in the inhibition of water absorption from the lower bowel of germfree rodents (10). This suggestion was substantiated by experiments which indicated that in conventional rats, nonabsorbable macromolecular compounds, when added either to the diet (polyethylene glycol, 11), or to the luminal perfusion fluid of their ligated cecal pouches (polyvinylpyrrolidone, 12) effectively inhibited water absorption. Increased levels of colloid water attraction and mild diarrhea, qualitatively similar to that seen in germfree rodents, was observed also in the lower bowel of conventional rats whose intestinal flora was partially inactivated by oral antibiotic treatment (13).

The purpose of this work was to study colloid osmotic pressure gradients between intestinal contents and the tissue component in their possible effect on the absorption of water from the lower bowel of germfree and conventional rats.

Materials and Methods. Intestinal contents were obtained from germfree (GF) and conventional (CV), Fischer 344 (Charles River CDF), 10–12 mo old male rats fed L-462 diet (14). The rats were anesthesized with pentobarbital sodium (35 mg/kg body wt) and killed by decapitation. On autopsy, cecal contents were harvested and subjected to the following: *A. Colloid osmotic pressure* (COP) *determinations.* (a) Basing on the results of preliminary tests (Table I), the supernatants of cecal contents were obtained by centrifugation at 30,000 *g* for 15 min. The liquid was pipetted off and the sediment discarded. (b) In one run of experiments (1. Series) the supernatants were prefiltered through 0.45 μmillipore filters (mainly for removing bacteria from the CV samples). In another run (2. Series) the supernatants were tested without prefiltration. The determinations of COP were carried out immediately following the steps a or b by a modification of the method of Krogh and Nakazawa (15). This entails in a tonometer the determination of the hydrostatic pressure which is able to counteract the

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[2] Present address: The Fukuoka National Higashi Hospital, Koga Kasuya-gun, Fukuoka, Japan 811-31.

TABLE I. PRELIMINARY OBSERVATIONS ON THE EFFECTS OF FILTRATION AND CENTRIFUGATION OF GF RAT CECAL SUPERNATANT ON COP (AVERAGE OF 3 RUNS EACH, NO ADDITIONAL MILLIPORE FILTRATION, COUNTERLIQUID 0.9% NaCl)

	COP in mmHg		
Filtration through glass wool	Centrifugation (15 min) at		
	1000 *g*	30,000 *g*	60,000 *g*
117	126	112	107

force of water attraction exerted by the colloid across a semipermeable membrane. The volume of the tonometer chamber was 0.5 ml, the membrane used was a cellulose dialysis membrane (thickness 0.028 mm, pore radius 24 Å, Union Carbide Corp., Chicago, IL). The counter-liquid was drawn up in double layer filter paper, which was placed underneath the dialysis membrane, resting on a perforated stainless steel disc. The counter-liquids used were either *1.* 0.9% NaCl solution, or *2.* ultrafiltrate prepared from the respective cecal content supernatants, using similar dialysis membrane. Each tonometer was supplied with a vertically placed glass capillary tube (i.d. 1 mm, length 20 cm) whose lumen was continuous with that of the tonometer chamber. On filling the tonometers with the supernatants (free from bubbles), the capillary tubes were inserted and tightly connected in such a fashion that the level of the supernatant reached approx. to the half mark of the capillary. Following these steps, the open ends of the capillaries were sealed with rubber caps and the tonometers were kept at refrigerator temperatures for 3 hr. Before taking the COP readings, the tonometers were allowed to warm to room temperature. At this point the caps were removed and the glass capillary was connected with a short piece of polyethylene tubing (PE 50) to a mercury manometer. All other details of the COP determinations were the same as given in the original description of the method. For each sample, triplicate or (in case the sample volume was limited) duplicate tonometers were set up. From the parallel readings of each supernatant (showing generally not more than 10% variation), arithmetic means and standard deviations were calculated. *B. Colloid water attraction.* In order to assay the ability of cecal supernatants to cause water movement when in contact with biological or related fluids across semipermeable membranes *in vitro*, the following complementary experiment was run. A modified Ussing chamber (16) of 6 ml volume was separated into two halves with the same type dialysis membrane as described under A (area of window was 3.8 cm^2). One half chamber was filled with cecal supernatant. The other half was filled with 2.5% polyvinylpyrrolidone (PVP mw 40,000, Sigma, St. Louis, MO) solution in saline. Both in the cecal supernatant, as well as in the counterliquids, the total osmolality was 290–310 mOsm. To each half chamber, a horizontally placed, calibrated capillary tube (similar in dimensions to the one described under A) was tightly attached in which air–liquid interfaces were established. This permitted the reading of volume changes in the half chamber's content without a buildup of hydrostatic pressure difference. Thirty minutes after the start of the experiment, the direction and the volume of liquid movement were recorded at 30 min intervals over a period of 3 hr.

Results. The preliminary observations indicated high values of COP in GF cecal supernatant (Table I). The mode of preparation of the samples (filtration through glass wool, centrifugation at various speeds) appeared to affect only slightly these values. Centrifugation at 30,000 *g* for 15 min was selected as most suitable. The main results of this study (Table II), confirming the preliminary findings, indicated that COP values in GF cecal supernatants were two to three times higher than in CV controls, irrespective whether saline or ultrafiltrate were used as counterliquids. In both GF and CV samples, when ultrafiltrate was used as counterliquid, COP readings were approx. 20% lower than in case when saline was used for this purpose. Prefiltration of the samples through a 0.45 μmillipore filter had no appreciable effect on the COP readings. Our complementary experiment on colloid water attraction indicated (Table III) that GF cecal supernatant drew liquid at a

TABLE II. COP (mmHg) OF RAT CECAL SUPERNATANT (30,000*g*, 15 MIN).[a]

Germfree			Conventional		
	counterliquid			counterliquid	
Rat No.	0.9% NaCl	ultrafiltrate	Rat No.	0.9% NaCl	ultrafiltrate
1. Series (0.45 μmillipore prefiltration)					
1	105	—	101	39	—
2	116	95	102	47	—
3	106	88	103	50	37
4	118	98	104	33	36
5	115	87	105	30	20
6	91	69	106	38	—
7	97	83	107	40	—
8	108	89	108	27	—
9	112	—			
M	108	87		38	31
SD	7	9		8	8
2. Series (no prefiltration)					
10	107	—	109	59	—
11	112	—	110	25	—
12	110	—	111	38	—
13	112	—	112	40	—
14	130	—	113	27	—
15	127	—	114	46	—
			115	48	—
			116	36	—
			117	46	—
M	116	—		41	—
SD	9			10	

[a] M = arithmetic mean; SD = standard deviation; — = no observation.

sizable rate from a PVP solution whose COP was approx 10 mmHg, and whose total osmolality was similar to that of the GF cecal supernatant.

Discussion. The COP values presently measured in GF cecal content supernatant are to our knowledge among the highest observed in the animal organism. As pointed out in the introduction, in this phenomenon the accumulation of mucus and of other macromolecular compounds appears to be implicated. This assumption is supported by observations which showed that in the effluent of GF cecal supernatant from a cationic exchange resin column (H^+ form), the peaks of titratable acidity and of hexuronic acid (characterizing in part acid mucopolysaccharides) coincided with those of viscosity and COP (12). The high levels of COP in the contents of the GF cecum in comparison to those of blood plasma (17) or of interstitial fluid result in a sizable gradient which appears to be sufficient to retain water in the cecal lumen, or even cause an efflux of water from the tissues into the lumen. The latter was indicated by the ability of GF cecal supernatant to draw water *in vitro* from a PVP solution whose COP was taken to be approx. equivalent to that of interstitial fluid. Water movement from the tissue into the GF cecal lumen *in vivo* is suggested also by an observation (8) which indicates that in GF mice the dry percent of ileal contents (27.5 ± 1.5), on entering the cecum, drops considerably (21.8 ± 1.2) whereas in CV controls, intestinal contents passing from the ileum into the cecum show an increment in dry percent (26.2 ± 1.7 and 31.5 ± 1.6, respectively). In addition to binding water, the excessive amounts of mucopolysaccharides in GF cecal contents appear to further reduce water absorption by sequestering cations (primarily Na^+) which are needed for the maintenance of solute coupled water absorption from the bowel

TABLE III. WATER ATTRACTION EXERTED BY GERMFREE RAT CECAL SUPERNATANT FROM PVP SOLUTION.[a]

Contents of half chamber No. 1 GF rat cecal supernatant		Contents of half chamber No. 2 2.5% PVP in saline		Water movement from half chamber No. 2 to No. 1
COP	Total osmolality	COP approx. 10	Total osmolality	4.4 ± 1.0 μl/cm²/
116 ± 9 mmHg	283 ± 6 mOsm	mmHg	300 ± 5 mOsm	hr

[a] Means and standard deviations of six experiments.

(12). In the development of these anomalies of GF rodents, the cecum is the primarily involved segment of the intestinal tract. The colon and the ileum appear to be less implicated in the phenomenon (8, 12). With progress of age, water absorption inhibition from the GF lower bowel, which is almost always combined with greatly increased contents and reduced intestinal muscle tone, worsen to the point that they constitute a major lesion observed at natural death of GF rodents (18).

In conventional animals, by reducing the cecal content's COP value close to that of blood plasma and by apparently freeing mucus-bound cations (12), the intestinal flora or some of its elements contribute to normal water absorption from the lower bowels. The identity of the involved flora elements is unknown. It is suspected that the implicated microorganisms act by way of supplying mucinases in the gut lumen (6). It is interesting to consider that in the course of evolution the rodent organism (and possibly other mammals) has failed to develop an adequate "colloid-degrading" mechanism in its intestine and is relying for this function, which is needed for the maintenance of the gut's physiologic normality on some of its microbial commensals.

Summary. Determinations of colloid osmotic pressure in the supernatant of germfree rat cecal contents indicated substantially elevated values in comparison to those of rat blood plasma or of conventional rat cecal supernatant. The germfree cecal supernatant, under conditions of similar total osmolality, was able to draw water at a sizable rate from a polyvinylpyrollidone solution whose colloid osmotic pressure was taken to be equivalent to that of interstitial fluid. It is suggested that the water absorption inhibition which was observed in the lower bowel of germfree rodents, is in part caused by the colloid osmotic pressure gradient which exists in these animals between the luminal contents and the tissue component.

1. Nuttal, G. H. F., and Thierfelder, H., Hoppe Seyler's Z. Physiol. Chem. **22,** 62 (1896–1897).
2. Combe, E., and Sacquet, E., C. R. Acad. Sci. Paris **262,** 685 (1966).
3. Evrard, E., Hoet, P. P., Eyssen, H., Charlier, H., and Sacquet, E., Brit. J. Exp. Pathol. **45,** 409 (1964).
4. Loesche, W. J., Proc. Soc. Exp. Biol. Med. **129,** 380 (1968).
5. Lindstedt, G., Lindstedt, S., and Gustafsson, B. E., J. Exp. Med. **121,** 201 (1965).
6. Hoskins, L. C., Gastroenterology **54,** 218 (1968).
7. Wilkins, T. D., and Gordon, H. A., Fed. Proc. **29,** 530 (1970).
8. Gordon, H. A., *in* "The Germfree Animal in Research" (M. E. Coates, ed.), pp. 127. Academic Press Inc., New York, N. Y. (1968).
9. Csáky, T. Z., *in* "The Germfree Animal in Research" (M. E. Coates, ed.), pp. 151. Academic Press Inc., New York, N. Y. (1968).
10. Gordon, H. A., and Nakamura S., Eighth Annual Meeting, Ass. Gnotobiotics, Oak Ridge, Tennessee, Abstr. No. 3 (1969).
11. Loeschke, K., Uhlich, E., and Halbach, R., Proc. Soc. Exp. Biol. Med. **142,** 96 (1973).
12. Gordon, H. A., and Wostmann, B. S., *in* "Biological Effects of Gnotobiotic Environment" (J. B. Heneghan, ed.), pp. 593. Academic Press, New York, N. Y. (1973).
13. Wiseman, R. F., and Gordon, H. A., Bacteriol. Proc. 1971, 114 (1971).
14. Wostmann, B. S., Ann. N. Y. Acad. Sci. **78,** 175 (1959).
15. Krogh, A., and Nakazawa, F., Biochem. Z. **188,** 241 (1927).
16. Ussing, H. H., and Zerhan, K., Acta. Physiol. Scand. **23,** 110 (1951).
17. Starling, E. H. "Principles of Human Physiology," p. 73. Lea and Febiger, Philadelphia (1956).
18. Gordon, H. A., Bruckner-Kardoss, E., and Wostmann, B. S., J. Gerontol. **21,** 380 (1966).

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