Anion Concentration in Cecal Content of Germ-free and Conventional Mice* (34070)

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In previous papers (1, 2), attempts were made to elucidate the mechanism for enlargement of the cecum in germ-free rats. A hypothesis was proposed that availability of permeable anion in the cecal content plays a crucial role in determining the cecal size. This hypothesis was based upon the following observations: (1) The chloride concentration in the cecal content of germ-free rats was extremely low compared to that of the conventional counterpart; (2) upon monocontamination with *Clostridium difficile* the chloride concentration increased more than ten times and the bicarbonate concentration four times, concomitant with cecal shrinkage; (3) by feeding anion-exchange resin in Cl form to germ-free rats, it was possible, without intervention of bacteria, to reduce the cecal size to one-half along with such changes in the composition of the content as reduced water content, decreased osmolarity, and increased chloride and bicarbonate concentration. Even the histological structure of the cecal wall lost some of the characteristic features of the germ-free cecum and approached those of the conventional rat.

The intestinal absorption of water is closely coupled to and dependent on the absorption of salts (3). Although the intestinal transport of chloride ion is mainly passive, at least *in vitro*, the absence of chloride or other permeable anions in the luminal solution can induce stoppage of active sodium transport and hence of water absorption. This situation is exemplified in the classical experiment of "chloride impoverishment" by Visscher *et al.* (4), in which the net transport of water is linearly related to the luminal concentration of chloride, and at the terminal stage absorption of water virtually ceases. The resemblance between the ionic compositon of germ-free cecal content and that of "chloride impoverishment" seem sufficient to account for the enlargement of the germ-free cecum without necessity of incriminating functional derangement in the mucous membrane. Further support is rendered by an almost reciprocal relationship between the cecal size and the chloride concentration observed in monocontamination and in Cl-resin feeding.

Although the mechanism is quite obscure for the low concentration of chloride in the germ-free cecal content and subsequent increase after infection, the operational importance of chloride or permeable anion seems well established in the control of cecal size in rats. In this paper, these observations are extended with mice in order to ascertain whether or not the concept propounded earlier is applicable in this species of rodent. Also analyses were made on the cecum of conventional mice that were fed with antibiotics, since this treatment was associated with a partial cecal enlargement (5). It is of interest to compare the ionic composition in the "temporarily germ-free" cecum of penicillin-treated mice with that found in genuine germ-free mice.

Method. Mice of CFW strain, either germ-free or conventional, were used without preference to sex at ages between 40 and 60 days. Germ-free mice were placed in a glass jar with steam-sterilized sawdust bedding in flexible plastic germ-free isolator. Diet L-462 was autoclaved at 122° for 25 min and fed

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along with sterile water *ad libitum*. Maintenance and care of the animals were performed according to the procedure established at Lobund Laboratory. Routine bacteriological test was performed to check the germ-free or monocontaiminated condition (6). The same diet and bedding were used for conventional mice.

Cl-resin diet. Rexyn 201 (Cl) chromatographic grade[1] was used as anion-exchange resin in Cl form. This is a resin of polystyrene-divinylbenzene alkyl quarternary amine type, with medium porosity and of mesh number 200–400. The exchange capacity is approximately 1 mEq/ml wet volume. The resin was thoroughly mixed with L-462 diet at 20% by weight and after addition of water at 15–20% the mixture was pelletized. Water was necessary to give the mixture the consistency needed for pelletizing. The resin diet was autoclaved in the same manner as for the control diet, and it was fed to weanling germ-free mice for 2 weeks.

Monocontamination. A strain of *Clostridium difficile* (ATCC no. 9689) was grown in fluid thioglycollate medium at 37° for 18 hr. The bacterial suspension was introduced to the mouth of germ-free mice with a cotton swab. In addition, the suspension was added to drinking water. Mice were sacrificed after 1, 3, and 6 days.

Penicillin G administration. Sterile potassium penicillin G, U.S.P.[2] was dissolved in distilled water at 0.1% and given to conventional mice in place of drinking water for 2 days.

Analyses. The handling of animals and detail of chemical analyses were similar to those already reported for rats (1, 2), except that, because of the small size of the mouse cecum, the cecal contents from 2–18 mice were pooled and analyses were performed on the pooled samples. The cecal weight relative to the body weight was calculated for each mouse. Centrifugation of the cecal content was performed with Sorval RC2-B centrifuge at 40,000*g* for 30 min at 2–5°.

Results and Discussion. Conventional mouse. Table I shows results obtained with individual samples of ceca in conventional mice. The characteristic features seen in the cecum of conventional rats are also recorded; namely, low percentage of water in the cecal content and cecal wall, hypertonicity of the content and moderately high concentration of chloride therein (2). The concentration of sodium seems to be higher than that in the rat cecal content, but the reason for this difference is not clear at present.

Germ-free mouse. Table IIA shows results obtained with ceca of germ-free mice. The peculiarity of the cecum of germ-free rats reported earlier (1, 2) with respect to the cecal size and the composition of the content is faithfully reproduced in germ-free mice. The cecal weight expressed on body weight basis is 10% compared to 2% in the conventional mouse. The high percentage of water reflects fluidity of the cecal content. The osmolarity and the concentration of sodium, potassium, chloride, and carbon dioxide have close similarity to those in germ-free rats reflecting the operation of a similar mechanism in inducing cecal enlargement.

Table IIB shows results obtained with germ-free mice fed with Cl-resin diet for 2 weeks. Effects of Cl-resin diet on cecal size, water percentage, and composition of the cecal content are identical to those observed with germ-free rats (2). The cecal weight decreased to one-half of the control value and the water percentage in the content de-

[1] By Fisher Scientific Co., Fair Lawn, New Jersey.

[2] Eli Lilly and Co., Indianapolis, Indiana.

TABLE I. Conventional Mice.

No. of mice	Cecal wt as % of body wt	% Water in cecal content	% Water in cecal wall	Osmolarity (mOsm/liter)	Na (m*M*)	K (m*M*)	Cl (m*M*)
14	1.59	74.5	71.0	392	76.2	22.9	27.4
15	2.24	72.4	70.2	470	145.0	—	17.6
16	2.21	73.2	73.4	456	100.0	14.7	16.5
16	2.26	73.6	70.4	404	126.5	19.7	17.4

TABLE II. Germ-Free Mice (A) and Germ-Free Mice Fed with Cl-Resin Diet (B).[a]

	Cecal wt as % body wt	% Water in cecal content	% Water in cecal wall	Osmolarity (mOsm/liter)	Na (m*M*)	K (m*M*)	Cl (m*M*)	CO_2 (m*M*)
A	10.4 ± 0.4	86.7 ± 1.4	75.0 ± 0.4	310 ± 3	56.9 ± 1.9	13.05 ± 0.79	3.65 ± 0.20	2.77 ± 0.27
	N = 41	*N* = 14	*N* = 14	*N* = 14	*N* = 14	*N* = 14	*N* = 14	*N* = 14
B	5.66 ± 0.09	67.1 ± 0.9	67.2 ± 0.8	268 ± 6	60.7 ± 3.4	18.68 ± 0.92	12.72 ± 0.48	—
	N = 81	*N* = 13	*N* = 13	*N* = 13	*N* = 11	*N* = 11	*N* = 13	

[a] Expressed as mean ± SE; *N* indicates the number of individual samples.

creased to a level comparable to semisolid appearance of the content. Also noteworthy is the response in osmolarity. As with resin-treated germ-free rats, the slight but definite hypertonicity of the control turned to hypotonicity. The chloride concentration showed a substantial increase.

Monocontamination. The data in Fig. 1 indicate changes induced by monocontamination with *Clostridium difficile* in germ-free mice. Only variables showing significant trends are depicted. The mouse cecum showed a small but definite enlargement above the control along with slightly increased concentration of chloride and carbon dioxide, in contrast to monocontaminated rats, which showed significant cecal shrinkage at this stage (1). It seems that bacterial invasion induces primarily an increase of concentration of anions, which in turn stimulates water absorption and cecal shrinkage. The peculiar cecal response of mice on the first day of infection may be explained as delayed onset of water absorption after the anion accumulation.

Subsequently the anion concentration reached maximum level on the third day, and the cecal size was much reduced. However, the anion concentration started to fall on the sixth day although the cecum remained reduced in size. It was reported that during prolonged observations on mice monocontaminated with *Salmonella typhimurium* the cecal size returned gradually to the control level of germ-free mice after a transitory shrinkage (7). The beginning decline in anion concentration observed on the sixth day may be an indication of later re-enlargement of the cecum.

The concentration of potassium showed a change similar to, but more gradual than the anion concentration. Although not depicted in the figure, the sodium concentration showed a very slow decline during the period of observation. Since it was shown in germ-free rats that the reduction of cecal size occurred on the first day of monocontamination without any change in potassium concentration, the increased concentration of potassium observed here may be due to inflammation in the mucosal membrane caused by

TABLE III. Conventional Mice Fed with 0.1% Penicillin G Solution for 2 Days.[a]

Cecal wt as % body wt	% Water in cecal content	% Water in cecal wall	Osmolarity (mOsm/liter)	Na (m*M*)	K (m*M*)	Cl (m*M*)	CO_2 (m*M*)
3.69 ± 0.08	82.4 ± 1.0	74.6 ± 0.6	367 ± 10	55.1 ± 2.4	12.89 ± 0.79	5.54 ± 0.30	5.00 ± 0.77
$N = 63$	$N = 10$	$N = 10$	$N = 10$	$N = 10$	$N = 10$	$N = 10$	$N = 6$

[a] Expressed as mean ± SE; *N* indicates the number of individual samples.

Clostridium and not directly concerned with the cecal size.

Penicillin administration in conventional mice. As indicated in Table III, cecal enlargement after administration of penicillin was similar to that reported by Savage and Dubos (5). The cecal weight increased to 1.5–2 times that of the conventional mouse, and the cecal content assumed marked fluidity as indicated in the increased water percentage. It is of interest to note that the changes observed in the composition of the cecum content after treatments with penicillin were analogous to those found in germ-free cecal even if to a lesser degree. The osmolarity of the cecal content became less hypertonic and the concentrations of sodium and chloride were decreased. These findings suggest that the mechanism for cecal enlargement by penicillin is similar to that operative in the germ-free cecum.

Savage and Dubos (5) emphasized the importance of fusiform bacterium in controlling the cecal size. However it has been clearly demonstrated that *Clostridium difficile* and *Salmonella typhimurium* were also effective in reducing cecal size in germ-free rodents, even if temporarily. Also the reduction in cecal size effected under sterile conditions by the addition of Cl-resin to the diet. From these observations it can be concluded that the availability of chloride or of permeable anions in the cecal content determines the size of the cecum; and the effectiveness of bacterial contamination in this respect seems to depend upon their ability to render chloride available for membrane transport.

Summary. Correlation between cecal size and the ionic composition of cecal content was determined in conventional mice, germ-free mice, germ-free mice fed with Cl-resin diet, germ-free mice monocontaminated with *Clostridium difficile*, and conventional mice treated with penicillin. Shrinkage of the cecum is best correlated with increased chloride and bicarbonate concentration; and enlargement of the cecum with decreased concentration. From these findings it was concluded that availability of chloride or permeable anion in the cecal content is the primary factor to control cecal size.

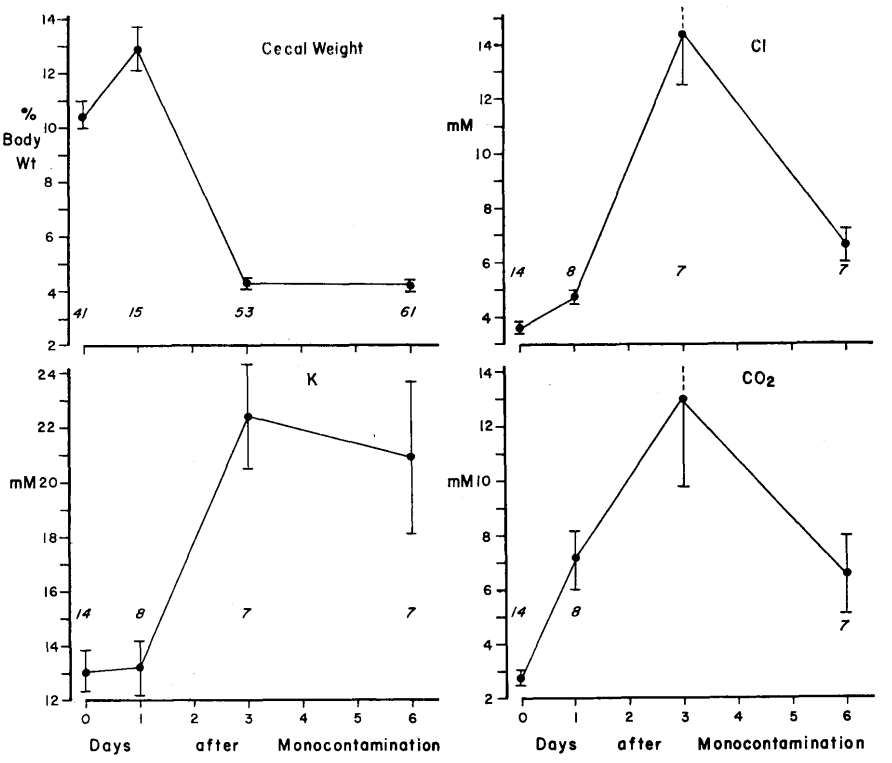

FIG. 1. Changes in cecal weight and composition of cecal content in germ-free mice after monocontamination with *Clostridium difficile*. The abscissa indicates time after contamination in days. The values on Day 0 indicate control values of germ-free mice without contamination. The ordinate indicates cecal weight as percentage of body weight, concentration of chloride, carbon dioxide, and potassium in cecal content in mM. The height of bar at each point indicates twice the standard error of the mean and the number in italics indicates the number of samples for each value.

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