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The Size, pH, and Redox Potential of the Cecum in Mice Associated with Various Microbial Floras (39187)

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Among the numerous metabolic and physical anomalies reported in the germfree rodent, the greatly enlarged cecum is perhaps the most obvious (2). The physical enlargement of the cecal sac and the altered physical and chemical character of its contents (as compared to values for conventionally reared rodents) cause several other dysfunctions, not all of which are corrected by cecectomy (6). The existence of these conditions suggests that the microbial flora contributes to the development of characteristics that are considered "normal" for conventionally reared rodents.

The normal bacterial flora of the laboratory rodent may be comprised of up to 500 distinct species (5). It seems reasonable to suggest that not all of these species play an equal and integral role in intestinal ecology. The possibility exists that a relatively simplified microflora could mediate normal rodent development. Such a microflora would most likely be derived from the obligately anaerobic population of the gut which predominates by at least 1000-fold over the facultative gut bacteria (4) in the lower gastrointestinal tract.

Past studies on the association of the obligately anaerobic bacteria with germfree rodents may have been hindered by the prevailing positive redox potential (Eh) within the germfree gut (1, 7). In the presented work, germfree mice were associated with various microbial floras. The degree of normalization of cecal size, pH, and redox potential was determined. The results indicate that with respect to the parameters measured, "normal" values can be observed in mice associated with simplified microfloras.

Methods. All animals used in this study were 2- to 6-month-old CFW mice maintained at Lobund Laboratory. With the exception of the conventional mice reared in the laboratory animal room, all mice were derived from the Lobund germfree CFW mouse colony and were housed under gnotobiotic conditions in Trexler-type plastic isolators. All mice were fed autoclaved L-485 diet (Teklad) and water *ad libitum*.

Mice were placed in the following five microecological groupings and the effects of the microflora on the size, pH, and Eh of the cecum were noted: (1) germfree, (2) conventional, (3) monoflora, (4) hexaflora, and (5) thermoduric polyflora. The monoflora group was made up of previously germfree mice monoassociated with *Clostridium* sp., a large spore-forming, cigar-shaped, gram positive tapered rod originally isolated from the cecal mucosa of a conventionally reared mouse. This organism is one of the predominant bacteria seen associated with the mucosal surface of the conventional mouse cecum. The hexaflora group consisted of *Streptococcus faecalis, Lactobacillus brevis, Staphyloccus epidermidis, Enterobacter aerogenes, Bacteroides fragilis* var. vulgatus, and a *Torulopsis* sp. The thermoduric polyflora group consisted of organisms derived from cecal wall homogenate that had been obtained from conventional mice and heated at 70° for 10 min. This flora was predominantly comprised of gram negative, fusiform-shaped bacteria, although other bacterial types were observed. All floras were introduced into experimental mice by oral and anal gavage.

Determinations of cecal size, pH, and Eh were made after a minimum of 3 weeks of association of the animals with the specified floras. Ceca were excised and weighed and relative weight was expressed as percentage of body weight.

Redox potential was determined in the following manner. Laparotomy was performed on anaesthetized mice (intraperitoneal sodium pentobarbital, 35 mg/kg) to expose the cecum. Electrodes were placed within the cecum and secured by sutures. A continuous 30-min measurement of the Eh

and pH was recorded. Care was taken to ensure a steady state by covering the cecum with gauze moistened with warm saline, and warming the area around the cecum with a tensor light beam.

Three electrodes were employed simultaneously. A platinum electrode (10 mil wire sealed in glass) was used to determine Eh recording against a silver–silver chloride wick–liquid junction reference electrode. The latter electrode also served as the reference electrode for pH measurements. The pH was measured with a micro glass electrode contained within a 16 gauge unbeveled hypodermic syringe needle (Micro Electrodes Inc., New Hampshire). Care was taken to clean the surface of the platinum electrode to prevent spurious readings (3). Prior to each determination, the platinum electrode and the recording system were calibrated against the standard hydrogen electrode and the voltage was taken as zero. The validity of redox potentials determined in this manner was occasionally verified by measuring the standard potentials of the ferrous-ferric chloride system, and the potassium ferri-ferrocyanide system.

The Eh was amplified by a Leeds and Northrup pH meter (Model 7401) and displayed on one channel of a Mosley two pen strip chart recorder (Model 7100A). The output of the pH glass electrode was amplified by a Leeds and Northrup pH meter (Model 7405) and displayed on the other channel of the recorder. The pH system was calibrated with Beckman standards.

Results. Table I illustrates the mean cecal weight of each group of mice expressed as percentage of total body weight. These data were obtained in a series of experiments separate from those in which pH and Eh were determined. However, the mice were treated identically. The data indicate that cecal size normalization in mice can be brought about by a simplified microflora (i.e., the thermoduric flora).

Records shown in Fig. 1 were obtained using mice that were maintained under germfree, conventional, or in monoassociated conditions. Steady Eh and pH were observed for a period of 30 min or more. For the conventional, and to some extent the monoassociated mice, approximately 10 min passed before a steady potential value was observed. This phenomenon possibly resulted from partial oxidation of materials immediately adjacent to the electrodes upon insertion.

Figure 2 is a composite diagram illustrating the temporal pattern of Eh in the cecal content of mice reared under one of five different microbial environments. Each point depicted represents the mean potential values obtained from six or more experimental runs. Germfree cecal contents expressed highly positive potential, thereby indicating an oxidizing environment in the cecum. By contrast, highly electro-negative Eh values were observed in conventional ceca indicating a reducing environment. The difference between germfree and conventional cecal Eh was greater than 400 mV, similar to results reported by Wostmann and Bruckner-Kardoss for germfree and conventional rats (8). Redox potential in the ceca of monoflora mice was close to zero. Potential in hexaflora and thermoduric-associated mice was indistinguishable from that observed in conventional mice.

Figure 3 illustrates the composite values of pH obtained simultaneously with the Eh values that were depicted in Figure 2. The pH does not show a definite tendency regarding gnotobiotic condition or temporal

TABLE I. MEAN CECAL SIZE EXPRESSED AS PERCENTAGE OF WHOLE BODY WEIGHT OF CFW MICE ASSOCIATED WITH SPECIFIED MICROFLORA.

Microflora associated	Cecal size (%) (mean ± S.E.)	P_1[a]	P_2[b]
Germfree	11.06 ± 1.60	–	<0.001
Conventional	1.64 ± 0.33	<0.001	–
Clostridium sp.	7.10 ± 2.20	<0.001	<0.001
Hexaflora	4.35 ± 0.90	<0.001	<0.001
Thermoduric	1.74 ± 0.17	<0.001	>0.40

[a] Confidence level of differences between germfree mean and the means of other groups listed.
[b] Confidence level of differences between conventional mean and the means of other groups listed.

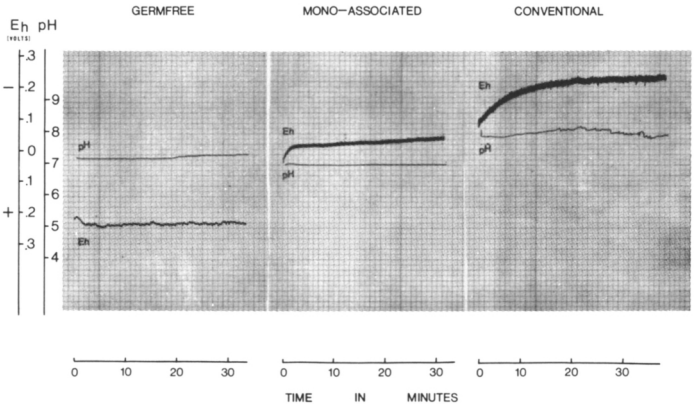

FIG. 1. Representative records of Eh and pH measured *in situ* in the cecal content of gnotobiotic mice. The ordinate indicates Eh in millivolts and pH, respectively, and the abscissa time in minutes.

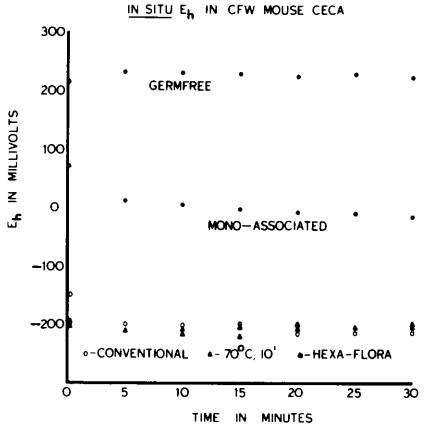

FIG. 2. Mean Eh values plotted at 5-min intervals for gnotobiotic mice. The ordinate indicates Eh in millivolts and the abscissa time in minutes.

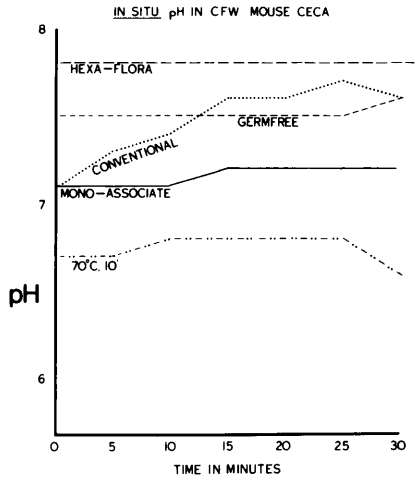

FIG. 3. Mean pH value plotted at 5-min intervals for gnotobiotic mice. The ordinate indicates pH and the abscissa time in minutes. The pH values were obtained together with Eh values presented in Fig. 2.

pattern. The small pH variations observed may reflect the varying metabolic patterns of the different microfloras. The pH difference between conventional and germfree ceca was 0.5 pH unit, which could account for a maximum of only 29 mV of the Eh difference observed (3). Therefore, the large difference in redox potential between germfree and conventional cecal contents must be attributed to differing oxidative and reductive conditions in those cecal contents.

Discussion. The germfree rodent is characterized by pecularities in anatomical as well as functional parameters which differ distinctly from those observed in their conventional counterparts. The greatly enlarged cecum observed in germfree rodents is the most outstanding structural anomaly. Upon exposure to external microbial environments, these parameters may revert to values characteristic of the conventional state, a process called normalization. By the introduction of specific microorganisms to germfree rodents, it is anticipated that degrees of normalization will be achieved, reflecting the normalizing ability of the associated microflora.

The question is raised as to whether normalization is induced in parallel for different parameters. The importance of this question lies in the possible interrelationship among a variety of parameters which could reveal causative sequences in the normalization process.

In this study, under a variety of gnotobiotic conditions, cecal size and redox potential were measured as indicants of the

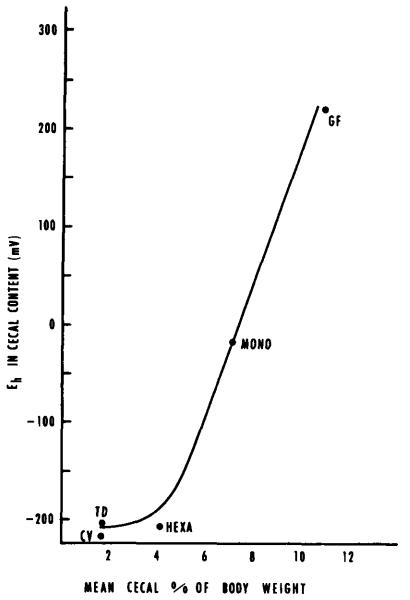

FIG. 4. Correlation between cecal size and Eh of cecal content in gnotobiotic mice. The abscissa represents the cecal size in percentage of body weight, and the ordinate represents the redox potential in millivolts. The meaning of symbols are as follows: GF, germfree; Mono, monoassociated; CV, conventional; TD, associated with thermoduric polyflora; Hexa, associated with hexaflora.

degree of normalization of test mice. In Fig. 4, the final (i.e., at 30 min) mean redox potential determined for each gnotobiotic condition was plotted against the corresponding mean relative cecal weight. The means of these values were used because data were obtained from different groups of mice under identical treatment. The linear relationship that would be expected if parallel normalization occurred was not observed. Since the curve is concave upward, redox potential is more easily normalized than cecal size. This conclusion is reasonable since the redox potential of cecal content is principally a function of microbial metabolism while cecal size is contingent upon complex functions such as transmural transport and the muscular tone of the cecal wall. It is unclear at present whether any causal relationship exists between cecal redox potential and cecal size.

The redox potential and cecal size of mice associated with the thermoduric polyflora are indistinguishable from those observed in conventional mice. This indicates the efficacy of the thermoduric polyflora in regard to the parameters chosen for this study. Further studies are in progress to determine whether other characteristics of conventional animals are assummed by mice associated with simplified microfloras. The isolation and characterization of the specific microbial members of the thermoduric polyflora are also underway.

Summary. Cecal size and *in situ* redox potential and pH of cecal contents were determined in conventionally reared mice and mice reared under a variety of gnotobiotic conditions: germfree, monoassociated with a cecal *Clostridium* sp., hexaflora-associated and thermoduric polyflora-associated. The mean Eh was approximately +200 mV in germfree and −200 mV in conventional mice. The Eh was close to zero in the monoassociated mice, thus occupying a position intermediate between the germfree and conventional mice. The potentials observed in the hexaflora and the thermoduric flora groups were indistinguishable from those of conventional animals.

The degree of normalization was more advanced with respect to the redox potential than to the cecal size in the various gnotobiotic groups. In the thermoduric polyflora-associated group, normalization was observed in both cecal size and redox potential. This demonstrates that normalization can be accomplished with a relatively simplified microflora, at least with regard to the parameters studied.

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