THE GASTROINTESTINAL EPITHELIUM AND ITS AUTOCHTHONOUS BACTERIAL FLORA*

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Comparative studies of the gastrointestinal microflora in various mouse colonies have been carried out for several years in our laboratory. Some of the findings, presented in earlier publications (1–5), can be summarized as follows:

(*a*) A few bacterial species reach high population levels in all mouse colonies tested, irrespective of the condition of husbandry, except in severe pathological states and of course in the germfree state. These bacterial species are anaerobic and persist throughout the whole life-span of the animal. In view of the general occurrence of these bacterial species, we have suggested that they evolve with their mouse host and constitute, therefore, its autochthonous flora (1).

(*b*) Whatever the genetic constitution of the animals, the composition of their so-called "normal flora" (i.e., the flora characteristic of the mouse colony) differs qualitatively from one colony to the other. It has been found, in particular, that the NCS and NCS-D mouse colonies, produced and maintained under protected conditions in our laboratory, have a much simpler microflora than that of the Standard Swiss (SS) colony from which they were derived. The bacterial flora of the gastrointestinal tract of the NCS and NCS-D colonies consists chiefly of the autochthonous species, in the sense described above (1–3).

(*c*) The various bacterial species, especially those of the autochthonous flora, do not occur randomly in the gastrointestinal tract, but rather exhibit marked selectivity in their anatomical localization. For example, autochthonous lactobacilli are extremely numerous in the stomach, where they exist as a well-organized layer on the mucosal epithelium. In contrast, the species of Bacteroides and fusiform bacteria do not occur in the stomach, but are extremely numerous in the large intestine (1, 3, 4).

(*d*) Under normal conditions, the various bacterial species colonize the gastrointestinal tract at different periods after birth, according to a sequence characteristic of each particular part of the tract (3, 5).

The conclusions outlined above were chiefly derived from the results of quantitative bacteriological analysis. In the present study, an effort was made to correlate these and other bacteriological findings with histological phenomena

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that occur in the various areas of the gastrointestinal tract at each stage of their colonization by the various bacterial species of the bacterial flora.

Materials and Methods

Mice.—The origin and characteristics of the NCS and NCS-D mouse colonies have been described in references 3 and 6. Other mice used were from the ordinary CFW colony of Carworth Farms (New City, N.Y.) and the specified pathogen-free, Caesarean-Originated, Barrier-Sustained (COBS) colony of the Charles River Farms Breeding Laboratories (Wilmington, Mass.).

In all experiments, the mice were given acidified water and commercial pellets essentially free of living microorganisms (see reference 6). Gravid females from the various colonies were maintained in individual cages containing wood shavings for bedding. The date of birth of the young was carefully recorded so that the experiments could be conducted with animals of precisely known age.

Preparation of Specimens for Bacteriological Examination.—Animals were sacrificed under chloroform anesthesia; the organs, always with their contents intact, were weighed and then homogenized in Teflon grinders in 5 ml of sterile charcoal water (2). Studies were carried out with the entire digestive canal from the esophagus to the rectum, or the whole stomachs, or segments of the small and large intestines.

Bacteriological Culture Techniques.—The homogenates described above were diluted in charcoal water in 10-fold steps. Calibrated loopfuls of each dilution were then spread on the surface of various selective agar media. The selective media and conditions of incubation used for the recovery and enumeration of lactobacilli, coliforms, enterococci, and anaerobes have been described elsewhere (2, 5).

Histological Techniques.—Segments of the intestines or the whole stomach with contents intact were frozen in a 2% solution of methyl cellulose (15 centipoise) in 0.15 M saline on the freezing shelf of a microtome-cryostat (International Equipment Co., Needham Heights, Mass.). Sections of the frozen tissues, cut at either 4 or 8 μ, were fixed for 60 sec in absolute methyl alcohol, and stained with hematoxylin and eosin, periodic acid–Schiff, or with a modified tissue Gram stain (7).

RESULTS

The Esophagus and Stomach.—As demonstrated previously, the bacterial flora that can be cultured with present techniques from the stomachs of NCS and NCS-D mice consists almost exclusively of lactobacilli and Group N streptococci (1, 3). These bacteria colonize the stomachs of infant NCS and NCS-D mice on the very first day after birth, and within 7 or 8 days reach high population levels that persist throughout normal life. In the present study, it was found that the stomachs of CFW and COBS mice also harbor populations of lactobacilli and Group N streptococci. These populations exist at the same levels and develop in infants in the same manner as that reported for the NCS mice. In addition, it was found that lactobacilli could also be consistently cultured in significant numbers from the esophagi of both infant and adult mice from the four colonies (Table I).

These findings were extended and their significance made apparent by histological studies. In histological sections, as early as the 2nd or 3rd day after birth, Gram-positive rods and streptococci could be seen in layers on the strati-

fied squamous epithelium of the nonglandular mucosa of the stomachs (Fig. 1) and the distal one-third of the esophagi of mice from the four colonies. In the stomach, the bacterial layer was confined to the keratinized epithelium and ended abruptly at the cardiac antrum. By the 7th to 10th day after birth, the layer was almost as well-developed as in the adult stomach (Fig. 2).

TABLE I

*Bacterial Flora of the Stomach and Esophagus of Infant and Adult NCS, NCS-D, CFW, and COBS Mice**

	Mice	Lactobacilli and Gr. N streptococci	Coliforms‡	Enterococci	Anaerobes§
Esophagus	NCS	10^7	±	±	±
	NCS-D	10^7	±	±	±
	CFW	10^7	±	±	±
	COBS	10^7	±	±	±
Stomach	NCS	10^9	±	±	±
	NCS-D	10^9	±	±	±
	CFW	10^9	±	±	±
	COBS	10^9	±	±	±

* The data are recorded as the average of the number of bacteria per gram of fresh tissue; 15 to 20 animals per group. The sign ± indicates occasional culture of very low numbers of bacteria that are probably not resident in the stomach or esophagus, but are passing through after being ingested during coprophagy. In infant mice, the lactobacilli and Group N streptococci become established in the gut within the first day after birth and increase to the levels shown by the end of the first week of life.

‡ The coliforms are predominantly slow fermenters of lactose (SLF) in NCS-D and NCS mice, but the latter also contain substantial numbers of lactose fermenters. Lactose fermenting coliforms are the predominant coliform flora in CFW and COBS mice.

§ The anaerobes include bacteroides and clostridia which can be cultured on agar media and also fusiform-shaped bacteria which have not yet been quantitatively cultured. On the proper anaerobic agar media, bacteroides constitute the most numerous organisms recovered from the mice of the various colonies tested; clostridia are recovered occasionally from the NCS and frequently from the CFW and COBS mice. From the 12th or 13th day after birth, fusiform bacteria outnumber all other bacteria, by a factor of 100–1000, as seen in the special histological sections of the cecums and colons of mice of the four colonies.

In a previous study, it was found, with NCS mice, that just as many lactobacilli and streptococci could be cultured from homogenates of the stomach mucosa washed three times with saline, as from the luminal contents and the washings of the mucosa (1). In the present histological study, few bacteria were seen free in the lumen, whereas the mucosal layers contained large numbers of Gram-positive rods and cocci (Fig. 3). These facts strongly indicated that the bacteria in the layers were lactobacilli and anaerobic streptococci.

The Small Intestine.—The types of bacteria that could be cultured from the

small intestines of the mice from the four colonies were essentially the same as were found in the stomach (Table II and reference 1). Again, lactobacilli and anaerobic streptococci appeared early and predominated throughout the lives of the animals. Histological views of an infant duodenum (Fig. 4) and an infant ileum (Fig. 5) are shown to illustrate the location and nature of the bacteria. As in the stomach, the bacteria that could be observed in the sections were Gram-positive rods and streptococci. In the small intestine, however, these bacteria were confined to the lumen; no layering on the epithelium was seen. In adults, the ileum had a more extensive bacterial population than any other area of the small intestine; but that population was composed only of Gram-positive bacteria.

TABLE II

*Bacterial Flora of the Small Intestines of Infant and Adult NCS, NCS-D, CFW, and COBS Mice**

Segment of intestine‡	Lactobacilli and Group N streptococci	Coliforms§	Enterococci	Anaerobes§
Duodenum	10^7	±	±	±
Jejunum	10^8	±	±	±
Ileum	10^9	±	±	±

* See the first footnote in Table I for the method of recording the data.

‡ The small intestine was arbitrarily divided into three segments. The duodenum was taken as a 1.5–3 cm piece just distal to the stomach; the jejunum and ileum were taken as the proximal and distal halves respectively of the remainder.

§ See the footnotes (‡, §) in Table I for a discussion of the various types of coliforms and anaerobes recovered.

The Cecum and the Colon.—It was reported in references 1 and 5 that the cecum and the colon of adult NCS mice contain a rich and varied bacterial flora, and that the various types of bacteria in this mixed population become established according to a certain time sequence in infants. These findings were confirmed in the present study for the NCS and NCS-D mice, and were extended to the CFW and COBS mice. During this sequential colonization, the period from about the 10th to the 18th day after birth was a time of profound rearrangement of the bacterial populations, as can be observed in Table III. The histological examination of the cecum and the colon made during that period provided further evidence of such readjustments.

As illustrated in Figs. 6–9, histological studies of the colon showed that the contents of the lumen contained mostly Gram-positive rods and streptococci until the infant mice were about 10 days old; the results were similar for the cecum. Around that time, microcolonies of tiny Gram-negative rods with rounded ends, and Gram-positive cocci, usually in pairs, appeared in the mucus

TABLE III

*Development of the Bacterial Flora in the Cecums and Large Intestines of NCS, NCS-D, CFW, and COBS Mice**

		Age of mice in days										
		2	4	6	8	10	12	14	16	18	20	60
Cecum	Lactobacilli and streptococci (Gr. N)	N	N	4	9	9	9	9	9	9	9	9
	Coliforms‡	N	N	N	N	±	6	5	5	5	4	4
	Enterococci	N	N	N	N	3	10	7	6	6	4	4
	Anaerobes‡	N(−)	N(−)	N(−)	N(−)	N(−)	6(1+)	8(2+)	9(3+)	10(3+)	10(3+)	10(3+)
Large intestine	Lactobacilli and streptococci (Gr. N)	±	4	8	9	9	9	9	9	9	9	9
	Coliforms‡	N	N	N	4	9	10	9	9	9	3	4
	Enterococci	N	N	N	6	9	9	9	8	8	±	4
	Anaerobes‡	N(−)	N(−)	N(−)	N(−)	±(−)	9(1+)	10(2+)	10(3+)	10(3+)	10(3+)	10(3+)

* The data are recorded as the average of the $\log_{10}$ of the numbers of the bacteria per gram of fresh tissue; 15–20 animals per group. The sign ± indicates bacteria infrequently cultured in low numbers; N, no bacteria recovered. The parentheses indicate the results of determinations from histological sections of the amounts of fusiform bacteria present; (1+) to (3+), small to enormous numbers; (±), infrequently observed in small numbers; (−), not observed.

‡ See the footnotes (‡, §) in Table I for a discussion of the various types of coliforms and anaerobes recovered.

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