Influence of *Salmonella typhimurium* on Ileum and Spleen Morphology of Germfree Rats[1]

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Introduction

Comparative studies in germfree and conventional chickens and rats by Gordon and Bruckner-Kardoss (4) and Abrams et al. (1) in mice, have indicated that the presence of a normal flora has an effect on the morphological characteristics of the intestine. Gordon and Bruckner-Kardoss (4) found that the germfree chicken generally had less lymphoid tissue in the ileum than did the conventional animal of the same age. Unpublished reports by Gordon also confirm this in male rats. Abrams et al. (1) showed that cellular renewal of ileal epithelium occurs at a much slower rate in germfree mice than in their conventional counterparts.

The effects of microbial flora on lymphatic tissue such as the spleen have recently been investigated by Bauer et al. (2) in mice; Thorbecke et al. (9) and Gordon (3) in chickens; Miyakawa et al. (6) and Sprinz et al. (8) in guinea pigs and Gustafsson (5) in rats.

The studies presented here extend the above observations to the rat which has been monoassociated with *Salmonella typhimurium* and more specifically to the effects this species has on the cellular elements of the intestine (ileum) and the morphology of the spleen.

Materials and Methods

Approximately 120 day old germfree female rats were orally seeded (as a monoassociate) with *Salmonella typhimurium* (strain 750). One group of animals was sacrificed after one day of association, while the other groups were sacrificed after 2, 3, 5, 8, 11 and 18 days. 120 day old germfree and conventional rats were also sacrificed as control animals. Animals were sacrificed by bleeding using nembutal anesthesia. The ileum and spleen were removed, embedded in celloidin and paraffin after the method of Apathy (7) and sectioned (5μ thick). Sections of the ileum were stained with hematoxylin, Azure II, and eosin, while the spleen sections were stained with methyl green pyronine.

Cellular elements were counted using a Net Reticule (American Optical Co.). The 100 mm^2 area of the reticule was divided into 0.5 mm squares. Using 970 x magnification only those nuclei of cells which "fell" under intersected lines of the 0.5 mm squares were counted. This procedure left very few cells unaccounted for. The cells of the villus and those of the areas of the submucosa and muscularis beneath the villus were counted. Three villi, selected at random, were counted for

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every animal and three or more animals were used for each determination. The arithmetic mean of these nine values are given in the table. These means are expressed as parts per thousand of the intestinal wall occupied by the listed tissue element.

Results and Discussion

Spleen Morphology.

Twenty-five mm^2 spleen sections of all animals were observed. The presence of bacteria (a complete flora or one species) did not seem to influence the number of follicles—the mean in all animals being approximately 18. Although follicles of both germfree and conventional animals varied greatly in size, the average dimensions were the same in both groups. In the monoassociated rats up to the fifth day of exposure the follicles were extremely large, thus leaving very little red pulp. After the fifth day they resembled those of the germfree and conventional animal. The presence of bacteria had little or no effect on the intermediate sinus. Germinal centers were observed in conventional, germfree as well as monoassociated animals.

The white pulp of germfree, conventional and monoassociated animals showed very little pyroninophilia in both inner and outer zones. The red pulp of monoassociates up to 5 days exposure resembled that of the germfree i.e. few pyroninophilic cells. After 5 days, however, extreme pyroninophilia was observed. Even after 18 days exposure to *S. typhimurium*, the red pulp still contained a large number of pyroninophilic cells.

Ileum Morphology.

A. Gross Changes

After the ileum of the germfree rat had been exposed to *S. typhimurium* for 2 days, there was a decrease in the number of villa. Epithelial cells were absent from the mucosal layer and thus the connective tissue was exposed to the action of the food, digestive enzymes and bacteria. Abrams et al. (1) found after treating the ileum of conventional mice with tritiated thymidine that the labeled epithelial cells reached the extrusion zone at the tip of the villus in approximately 2 days. It is conceivable therefore that after a germfree rat has been challenged by *S. typhimurium* for 2 days, that cellular proliferation in the crypts does not keep pace with epithelial loss from the extrusion tip; the result—a loss of epithelial cells lining the lumen and ultimately a partial or total destruction of the villus.

Five days after exposure the number of villi was "normal" but the epithelium of the villi was frequently separated from the underlying connective tissue. This separation may be a physical phenomenon wherein during the repair phase connective tissue proliferation does not keep pace with increased epithelial proliferation and migration, or it is possible that this represents a "physiologic inflammation." From the first to the fifth day of exposure there was a general increase in the number and length of the crypts of Lieberkuhn. After the eighth day the crypts and villi appeared "normal" both in length and number, resembling at this time those of the ileum of conventional rats.

B. Cellular Changes

TABLE I

Distribution (parts per thousand) of cells of the villi and of adjacent submucosa and muscularis layers of the ileum of 120 day old germfree, conventional and *Salmonella typhimurium* associated female rats. Each figure represents the mean from 9 or more randomly selected areas, from three or more different microscopic preparations (animals). S.D.M. values given.

	Germfree	Conv.	Days after Exposure to *Salmonella typhimurium* 1	3	5	8	11
Epithelium							
Villus epithelium	288 ± 16.8	199 ± 22	195 ± 26.1	103 ± 40.1	140 ± 16.6	147 ± 21.5	160 ± 24.8
Lieberkuhn epithelium	97 ± 14.3	124 ± 10.5	188 ± 20.4	245 ± 22.8	283 ± 20.5	218 ± 20.5	230 ± 8.1
Goblet	34 ± 7.5	25 ± 4.7	15 ± 6.8	2 ± 0.4	10 ± 5.2	6 ± 0.7	8 ± 1.0
Lymphocytes	2 ± 0.1	6 ± 1.1	2 ± 0.4	0+ ± 0.4	1 ± 0.4	1 0	1 0
Schollen leucocytes	1 ± 0.1	1 ± 0.2	0 0	0 0	0+ ± 0.9	0 0	1 0
Lamina propria and Submucosa							
Conn. elements	151 ± 21.6	137 ± 6.6	128 ± 31	206 ± 37.0	194 ± 15.8	248 ± 9.4	180 ± 7.4
Lymphocytes	13 ± 4.6	52 ± 6.9	5 ± 0.3	8 ± 0.4	11 ± 2.2	14 ± 4.1	9 ± 0.1
Ring	3 ± 1.0	4 ± 0.2	6 ± 5.5	9 ± 2.6	2 ± 0.4	1 ± 0.4	5 ± 2.5
Eosinophils	4 ± 1.3	10 ± 2.1	3 ± 0.2	2 ± 0.4	6 ± 2.2	2 ± 0.4	4 ± 1.0
Mast	0+ —	1 ± 0.2	1 ± 0.5	0+ ± 0.4	5 ± 1.3	0 0	0+ —
Plasma	1 ± 0.1	16 ± 7.3	4 ± 1.8	3 ± 0.6	1 ± 0.6	8 ± 2.7	6 ± 1.0
Muscle	5 ± 2.3	6 ± 1.8	26 ± 3.1	20 ± 4.8	15 ± 0.7	12 ± 2.3	14 ± 1.1
Muscle							
Circular	263 ± 18.6	251 ± 15.2	291 ± 16.8	251 ± 14.0	235 ± 20.0	230 ± 89.9	230 ± 6.0
Longitudinal	142 ± 10.9	159 ± 16.7	134 ± 9.2	152 ± 32.5	102 ± 3.8	113 ± 4.9	148 ± 7.2

The distribution of cells in the ileum of germfree, conventional and monoassociated rats is given in Table I. The germfree and conventional cell counts are generally of the same order of magnitude as Gordon (unpublished data) obtained with male rats on diet L-356. Gordon, however, did find many more lymphocytes and Schollencytes in the epithelial layer of the conventional rats than in the corresponding part of the germfree. Results in the table show that there are only 3 x as many lymphocytes in the epithelial layer of the conventional as in that of the germfree rat. The difference between Gordon's and these results may possibly be due to diet, sex or to both.

The number of villus epithelial cells of all monoassociates is less than either germfree or conventional; however, the Lieberkuhn cells increase, reach their peak the fifth day after exposure and then decline. Goblet cells are fewer in number in all monoassociates as compared to both germfree and conventional. Schollen leucocytes of germfree, conventional and monoassociates are of the same order of magnitude.

The connective tissue elements of the lamina propria and the submucosa increase up to the eighth day of exposure and then decline. The number of lymphocytes remains approximately the same as germfree and never equals the number found in the conventional rat.

Ring cells reach a peak on the third day; eosinophils on the fifth day and plasma cells on the eighth day after exposure. Monoassociates have many more muscle cells in the lamina propria and muscularis mucosa than either germfree or conventional rats.

There appears to be no appreciable difference in the muscularis layers of germfree, conventional and monoassociated animals. It is to be noted, however, that in all three groups the circular muscle layer is approximately twice as thick as the longitudinal.

These observations seem to indicate that most of the significant histological changes in the ileum and spleen (with limited data for this organ) occur between the second and the eighth day after exposure to *Salmonella typhimurium*. It appears that these changes (accompanied by or preceding phagocytic activity and antibody formation) have occurred to meet the challenge of the invading bacteria.

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