RELATION OF THE INDIGENOUS FLORA OF THE SMALL INTESTINE OF THE RAT TO POST-IRRADIATION BACTEREMIA

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There is general agreement that bacteremia occurs as one sequel to midlethal doses of whole body x-irradiation (Warren and Whipple, 1923; Mottram and Kingsbury, 1924; Chrom, 1935; Lawrence and Tennant, 1937; Bennett et al., 1949; Miller et al., 1951 Gonsbery et al., 1953). This infection could occur as a terminal event in animals dying of radiation injury; however, the findings of Miller et al. (1951) support the view that the infection once initiated usually leads to death and is not an agonal event. This is further supported by the finding that treatment with antibiotics (Miller et al., 1950; Smith et al., 1953) reduces mortality from x-radiation, and by findings reported here.

The bacteremia which develops after x-irradiation may be directly related to the failure of the animal's normal defense mechanisms against bacterial invasion. However, post-irradiation changes in the intestinal flora may also be of considerable significance to an understanding of the mechanism of development of the septicemia.

This paper reports a study of changes in the flora of rat small intestine following whole body x-irradiation. The findings suggest an interrelationship between indigenous pleomorphic lactobacilli in the small intestine and the invasive gram negative bacteria associated with the post-irradiation bacteremia. The finding of this interrelationship has led to the recognition of antimicrobial activity associated with the intestinal lactobacilli of the rat.

METHODS

Mature albino rats from two sources, the University of California at Los Angeles Atomic Energy Project colony and from Carworth Farms, were used. The Carworth rats were adapted to conditions of temperature and diet in the project vivarium for 2 to 8 months before use. All rats received the Rockland rat diet supplemented with a small amount of fish oil. Rats were irradiated, usually in groups of 30, by exposure to single total-body doses of 650 r delivered at 250 kv, 15 ma, at a distance of 100 cm (F.O.D.), using 0.21 mm inherent Cu plus 0.5 mm parabolic Cu and 1.0 mm Al filter, at approximately 10 r per minute.

Samples for bacteriological examination were generally taken from pooled samples from groups of three rats and were obtained with aseptic procedures from animals anesthetized with ether. Heart blood was drawn without anticoagulants and plated directly. Liver, spleen, and kidneys were pooled, weighed, immediately cooled to minimize post-mortem bacterial growth, and homogenized in sterile isotonic saline in a Waring blender. Sterile saline dilutions of the homogenates were plated. Dilutions of homogenized gut were prepared similarly and represented the small intestine from pylorus to ileo-cecal valve.

All samples were plated in triplicate, and counts were made by standard plate counting techniques. Plating was made routinely on nutrient agar, tryptose agar (Difco), and liver-veal agar (Difco). As aids in the estimation of the relative concentrations of the bacterial groups present, and to obtain counts of organisms which were present in inadequate numbers for satisfactory plating of the usual dilutions, the following differential media were employed: mannilitol salt agar (Difco), bromthymol blue lactose agar (Difco), Levine eosin methylene blue agar, S. S. agar, mitis salivarius agar (Difco), tomato juice agar (Difco), and Brewer's anaerobic agar. Bacteria from representative colonies were further characterized by staining and by their cultural characteristics noted in appropriate subcultures. Filterable forms of the pleomorphic
lactobacilli were recognized by subculture of material which passed through ultrafine fritted glass filters, Selas or Berkfeld bacteriological filters.

Antibacterial activity was assessed qualitatively on poured plates of 25 ml liver veal real agar containing approximately 300 colonies per plate. The plates were aged for 1 and 3 weeks before use. Streaks of test organisms were made on the surface, and the plates incubated and observed for growth after 10 to 48 hours. Viability of test organisms in the streaks were determined by subculture on appropriate media.

RESULTS

Normal flora of the rat small intestine. A microorganism indigenous to the small intestine of the rat was recovered in large numbers. The organism was found to be gram positive, nonmotile, pleomorphic, and microaerophilic. Sucrose, glucose, and lactose were fermented with the production of acid but no gas, and under reduced oxygen tension lactic acid was produced. Degeneration forms and variability in gram staining were observed frequently. On the basis of these characteristics the microorganism was presumed to be a strain of Lactobacillus acidophilus. A more detailed characterization of this lactobacillus will be presented later.

On the basis of cultural characteristics and tissue invasiveness, all strains of pseudomonads isolated from the intestine and other tissue samples were considered to be strains of Pseudomonas aeruginosa.

The preponderance of the coliform isolations was unable to ferment lactose or showed an adaptive delay and accordingly was classified as genus Paracolobactrum. Other coliform organisms with characteristics of Escherichia coli, E. freundii, and E. intermedium were found in small numbers.

Proteus vulgaris and Micrococcus pyogenes var. aureus were usually recovered from the small intestines in concentrations of less than 10,000 per gram of intestine. Enterococci, Streptococcus mitis, and aerobic sporeforming bacilli were occasionally noted in small numbers on differential agar media. A yeast resembling Saccharomyces cerevisiae, whose varying numbers in the counts were thought to be an expression of its viability in the rat food, was the only organism of these noted above which could be traced to the diet.

The normal flora of the small intestine of rats from the two sources were similar; however, strain differences were noted and characterized by the comparatively rough and dry growth of the coliforms and by the slow growth and paucity of pigment production of the pseudomonas organisms from the Carworth rat. These rats had a consistently larger gram negative flora than the project colony. The lactobacilli from the Carworth rats differed from the lactobacilli from project colony rats in producing smaller colonies with shorter periods of viability on plating agar, in their greater tendency to change into tiny, filterable forms on subculture, and in their inability to ferment xylose.

The post-irradiation flora of the small intestine and the bacteremic invasion. Bacterial populations in the flora of the small intestine through the various stages of the post-irradiation syndrome are presented in table 1. The rats selected as samples of a particular post-irradiation period were chosen as representatives of the period and included no obviously moribund animals.

The predominating type of intestinal bacteria in the normal rat is the pleomorphic lactobacillus. The numbers of this organism decline during the post-irradiation period and reach a minimum about the 7th day. The numbers of invasive pseudomonads are normally small, are tripled in number by the 4th post-irradiation day, and reach a maximum of a 1,000 times the normal number about the 7th day. The numbers of coliform organisms also increase in the post-irradiation period but appear to reach a maximum somewhat earlier than do the Pseudomonas species. The Proteus species show variable increases.

The counts obtained for normal rats show a considerable range, and this is even more extreme in the post-irradiation specimens. The scatter was considered in part a reflection of the variation in severity of the post-irradiation syndrome within the group from which rats were sampled. Accordingly, populations of lactobacilli and pseudomonads were measured in groups of rats separated on the basis of the severity of their post-irradiation symptoms. On the 7th day following a total body dose of 650 r rats were separated into two groups, those showing extreme symptoms (ruffled pelt, marked diarrhea, pronounced nasal and ocular hemorrhage, and ataxia) and those visually exhibiting less marked
TABLE 1

Bacterial populations in small intestine and in combined liver, kidney, and spleen homogenates of the rat following 650 r of x-radiation. Data are for rats from the Project colony. Counts are expressed as numbers of bacteria per gram of fresh tissue.

<table>
<thead>
<tr>
<th>Post-Irradiation Period</th>
<th>Tissue</th>
<th>No. of Samples (Pooled Groups of 3)</th>
<th>Lactobacilli Mean (Range)</th>
<th>Pseudomonas Mean (Range)</th>
<th>Coliforms Mean (Range)</th>
<th>Proteus Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\times 10^{4}$</td>
<td>$\times 10^{3}$</td>
<td>$\times 10^{3}$</td>
<td>$\times 10^{4}$</td>
</tr>
<tr>
<td>Control</td>
<td>Intestine</td>
<td>4</td>
<td>117 (60-140)</td>
<td>0.025 (0.012-0.050)</td>
<td>0.023 (0.01-0.05)</td>
<td>0.14 (0.50)</td>
</tr>
<tr>
<td></td>
<td>Liver Kidney Spleen</td>
<td>4</td>
<td></td>
<td>0.0*</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3-4 days</td>
<td>Intestine</td>
<td>4</td>
<td>52 (1.5-95)</td>
<td>0.290 (0.06-0.50)</td>
<td>4.6 (0.50-10)</td>
<td>5.2 (0-10)</td>
</tr>
<tr>
<td></td>
<td>Liver Kidney Spleen</td>
<td>4</td>
<td>1.1 (0-2.0)</td>
<td>0.0</td>
<td>12.0 (2-40)</td>
<td>0.3 (0-2.0)</td>
</tr>
<tr>
<td>6-7 days</td>
<td>Intestine</td>
<td>5</td>
<td>27 (1-55)</td>
<td>22.0 (3.0-50)</td>
<td>0.72 (0.10-1.0)</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Liver Kidney Spleen</td>
<td>4</td>
<td>180.0 (10-800)</td>
<td>2,300 (180-5,000)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10-14 days</td>
<td>Intestine</td>
<td>4</td>
<td>32 (1-100)</td>
<td>0.56 (0.03-2.0)</td>
<td>0.14 (0.03-0.30)</td>
<td>3.3 (0.4-10)</td>
</tr>
<tr>
<td></td>
<td>Liver Kidney Spleen</td>
<td>4</td>
<td>13.0 (0-90)</td>
<td>19.0 (0.0-90)</td>
<td>0.2 (0-0.5)</td>
<td>0.06 (0.0-0.5)</td>
</tr>
<tr>
<td>30 days</td>
<td>Intestine</td>
<td>2</td>
<td>153 (120-210)</td>
<td>0.026 (0.012-0.40)</td>
<td>0.025 (0.01-0.04)</td>
<td>1.8 (0-3.6)</td>
</tr>
<tr>
<td></td>
<td>Liver Kidney Spleen</td>
<td>2</td>
<td>0.5 (0-2.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Filterable forms were present in small numbers.
TABLE 2

Bacterial populations in small intestine and in combined liver, kidney, and spleen homogenates of 7th post-irradiation day rats grouped according to the severity of their post-irradiation symptoms. Counts are expressed as numbers of bacteria per gram of fresh tissue. Data are for rats from Carworth Farms.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue</th>
<th>No. of Samples (Pooled Groups of 3)</th>
<th>Lactobacilli Mean (Range)</th>
<th>Pseudomonas Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>× 10⁻⁴</td>
<td>× 10⁻³</td>
</tr>
<tr>
<td>Control</td>
<td>Intestine</td>
<td>4</td>
<td>1.17</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(60-140)</td>
<td>(0.012-0.05)</td>
</tr>
<tr>
<td></td>
<td>Liver, kidney, spleen</td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Extreme symptoms</td>
<td>Intestine</td>
<td>3</td>
<td>1.39</td>
<td>3.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.39-3.5)</td>
<td>(0.38-10.0)</td>
</tr>
<tr>
<td></td>
<td>Liver, kidney, spleen</td>
<td>3</td>
<td>19.4</td>
<td>5140</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.0-67)</td>
<td>(810-9,000)</td>
</tr>
<tr>
<td>Minimal symptoms</td>
<td>Intestine</td>
<td>3</td>
<td>108</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(84-148)</td>
<td>(0.018-0.06)</td>
</tr>
<tr>
<td></td>
<td>Liver, kidney, spleen</td>
<td>3</td>
<td>3.45</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.00-9.0)</td>
<td></td>
</tr>
</tbody>
</table>

damage (ruffled pelt, slight diarrhea, and minimal nasal and ocular hemorrhage). Counts were made as before on samples of small intestine and mixed liver, kidney, and spleen. These data are presented in table 2.

It is evident that animals showing the most marked radiation effects have smaller numbers of pleomorphic lactobacilli and much larger numbers of pseudomonads in the gut than do the rats which appear to be less ill. The differences are quite marked considering the subjective manner by which the animals were grouped.

The bacteremia existing in those rats whose intestinal populations were studied was also evaluated. Aliquots of mixed spleen, liver, and kidney homogenates were plated and counted, and these counts used as a measure of the bacterial invasion. These data are included in tables 1 and 2. Counts of blood borne bacteria were made but are not reported since positive cultures were obtained only in these instances where the liver-kidney-spleen count was very high.

The normal rat appears to have a minimal bacteremia which is confined to the pleomorphic lactobacilli. Of these, filterable forms are always present in small number, and occasionally large forms are found. Following x-irradiation the numbers of lactobacilli increase in the mixed spleen, liver, and kidney homogenates. Probably of more serious consequence is the appearance of disastrous numbers of coliform and Proteus species by the 3rd and 4th days and pseudomonads by the 6th and 7th days. Rats surviving beyond this interval have small counts of gram negative organisms, and 30 day survivors are abacteremic except for small numbers of pleomorphic lactobacilli. The only filterable forms found in the post-irradiation period are lactobacilli.

The severity of the post-irradiation syndrome is reflected in the extent of the bacterial invasion. In rats showing extreme symptoms the lactobacilli in combined homogenates of liver, kidney, and spleen are somewhat elevated over those from rats showing minimal symptoms. Far outweighing in significance this moderate invasion by lactobacilli are the huge numbers of pseudomonads found in the tissues from rats evidencing extreme post-irradiation distress. In rats showing minimal symptoms the distribution of pseudomonads in the gut and the liver-kidney-spleen homogenates is quite similar to the normal distribution.

Antimicrobial activity associated with rat intestinal lactobacilli. The striking reciprocal relationship between the populations of lactobacilli and
Cultures of lactobacilli, isolated from rat intestine and cultured on liver-veal agar, develop by 3 weeks, but not by 1 week, marked bactericidal activity against several representative gram negative and gram positive organisms. No evidence could be found that the antimicrobial effects were due to changes in pH, exhaustion of essential nutrients, or formation of peroxides by the lactobacilli.

Considerable study has been given the antibacterial activity associated with the lactobacilli, and the results of these studies will be presented later.

**DISCUSSION**

Studies of the flora of the small intestine of a variety of species, principally concerned with the relation of diet to the microbial population, have been reviewed fairly recently by Johansson and Sarles (1949). Of the small laboratory mammals, the microflora of the rat small intestine has been studied by Porter and Rettger (1940) and that of the mouse by Gall et al. (1948). Miller et al. (1951) have studied the normal flora of the mouse intestine in relation to the post-irradiation bacteremia.

There has been general agreement that lactobacilli constitute the predominant flora of the small intestine or that they are present in considerable numbers. The population of lactobacilli found in this study is higher than that reported for the rat by Porter and Rettger (1940). This may reflect a strain difference or equally likely a difference in sampling procedure. Porter and Rettger employed lumenal sampling while here the entire small intestine, as a homogenate, has been sampled to permit recognition of organisms sequestered in crypts of the intestinal mucosa.

Several mechanisms may be suggested to explain the decline in numbers of lactobacilli and the increase in pseudomonads and the coliform species in the post irradiation rat. The most attractive hypothesis is suggested by recent experiments in this laboratory, on gastric motility in irradiated rats.

In rats given median lethal doses of x-irradiation, gastric emptying time was greatly impaired beginning immediately after whole body irradiation as evidenced by the rate of passage of a barium sulfate meal. Further, the normal emptying of the stomach did not resume until the 7th day after irradiation (Billings, 1954, personal communication). Porter and Rettger (1940)
found that diet altered the flora and specifically that the lactobacilli markedly declined on a 5 day fast. The decrease in food supplied to the intestine, due to impaired gastric motility during the first seven days after irradiation, could account for the coincident decrease in intestinal lactobacilli.

Because of the antimicrobial activity associated with the lactobacilli, it seems likely that a natural antagonism exists in vivo between the intestinal lactobacilli and the other indigenous intestinal organisms. Assuming such a natural competition, a decrease in the numbers of lactobacilli would permit an increase in coliform and *Pseudomonas* species. In an animal in which the normal defense barriers have been damaged by irradiation, the numbers of coliform and pseudomonads might well attain the levels reported in table 1.

The enteric origin of the bacteremia following whole body irradiation has been adduced by Lawrence and Tennant (1937), Miller et al. (1951), and Furth et al. (1952), among others. Miller et al. (1951) suggest that the cecum is the probable source of the post-irradiation bacteremia in mice. That the small intestine is a major source for the post-irradiation bacteremia in the rat is suggested by the parallelism between the quantity and kind of bacteria in the small intestine, and the quantity and kind of organisms in the bacteremic samples. Contributions may also come from the large intestine. Factors which favor migration of specific bacteria from small intestine to other organs of the animal may include their concentration at suitable points of entry, their motility, and the capacity to elaborate tissue-damaging fractions.

The avenue of migration from the intestine to other organs of the rat is still under investigation. The mechanism by which the post-irradiation bacteremia is achieved appears to differ from the transmural migration of *E. coli* into the peritoneal cavity produced experimentally by Schweinburg et al. (1950), by chronic chemical irritation of the intestinal serosa. The peritoneal cavity of our irradiated rats was sterile since aseptic swabblings of the peritoneal cavity produced no growth when the swabs were incubated in broth.

Furth et al. (1952) suggest that "damaged capillaries and ulcerations of the intestinal mucosa, damage of the reticulo-endothelial barriers, destruction of mesenteric lymph nodes and leukopenia" may be basic factors in the spread of the enteric infection. The early healing of the intestinal tract after irradiation (Warren and Whipple, 1923) and often before bacteremia may be detected in the animal continues to puzzle investigators of the enteric migration. The relationship between flora of the small intestines and the bacteremic population found here indicates that the small intestine may continue to furnish a source for the bacteremic invasion throughout the acute phase of the post-irradiation syndrome.

Bacteremia is most severe during the period of greatest mortality, a relationship that has been particularly well established by the work of Miller et al. (1951). It may be noted from tables 1 and 2 that the gram negative bacterial invasion of the small intestine had reached its peak in the rats about the 7th day after irradiation. This period coincides with the predominance of *P. aeruginosa* in the bacteremic samples as well. Mortality in our unsacrificed, irradiated rats was definitely at its height on the 7th day. Miller et al. (1952) found that his strains of mice were normally free from *P. aeruginosa*, but that the contamination of his mouse colony with pseudomonas caused a sharp rise in irradiation mortality and a sharp decline in the effectiveness of the antibiotic treatments of the mice. Marston et al. (1953) found a *Pseudomonas* species the most virulent of the microorganisms tested in irradiated mice. It appears to be no coincidence that the peak mortality in the rat corresponds with the invasion of *P. aeruginosa*.

That antimicrobial relationships exist between indigenous animal flora more commonly than generally supposed is supported by a number of recent reports. Halbert (1948) has found coliform bacteria which produce antagonistic material; Halbert and Swick (1952) have reported antibiotic producing bacteria in the ocular flora; Evans et al. (1950) have discovered antagonisms in the flora of the skin; while Scrivener and co-workers (1950) have found considerable evidence of antagonisms among bacteria from the oral cavity.

White and Hill (1949) have reported the isolation of a morphological variant of *L. acidophilus* from saliva which not only inhibited the growth of *A. aerogenes* but also inhibited the growth of other lactobacilli, and finally Wheater et al. (1951) have obtained "Lactobacillin" active against *M. pyogenes* var. *aureus* from a strain of lactobacillus isolated from Gruyere cheese.
SUMMARY

The predominant microorganism in the small intestine of the rat is a lactobacillus. It is accompanied by smaller populations of pseudomonas, proteus, and coliform organisms.

Following 650 r of x-irradiation the numbers of lactobacilli in the small intestine fall to one-fourth their normal numbers. The pseudomonads increase about 1,000-fold by the 7th day. The proteus organisms and coliforms show less marked increases.

The invasion of the small intestine by gram negative organisms is paralleled by a bacteremia of types characteristic of the gram negative invaders in the gut. The severity of the invasion of the gut and tissues appears to correlate with the severity of the post-irradiation syndrome.

A natural antagonistic relationship between the indigenous lactobacilli and the gram negative strains normally present in only low numbers in the small intestine is supported by the finding of antimicrobial activity against these organisms in cultures of the lactobacilli.

REFERENCES


