

# NOTES ON INTESTINAL FLORA IN THE TROPICS

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The relative frequency of intestinal disorders in tropical countries, the multiplicity of microorganisms to which a causal relation has been attributed, the uncertainty prevailing about the distribution and significance of such bacteria as the Morgan bacillus and various *Salmonella* types and the general lack of knowledge about the etiology of tropical intestinal disease combine to give to even fragmentary observations some degree of value in guiding further research. In the course of various studies on other matters in Puerto Rico and in the Panama Canal Zone, we have gathered a few data upon intestinal flora which it seems worth while to place on record.<sup>1</sup>

Enteritis and diarrhea are common in Puerto Rico. On this island in 1930 (January to March), 144 fecal specimens were examined by the poured plate method; these specimens were derived from 81 persons. They were distributed as follows:

Persons (mostly children) with "enteritis," "diarrhea," etc. . . . . .	58
Hospital patients (accidents, heart disease, diabetes, etc.) with no gastrointestinal symptoms . . . . .	11
Apparently healthy persons . . . . .	7
Hospital patients with diagnosis of "sprue" . . . . .	5

Specimens were obtained in fresh condition by messenger service. Platings were made direct on Endo medium and also after one, two and sometimes three days incubation of pea-sized amounts in brilliant green bile medium. Glycerol as used by Teague and by Straus<sup>2</sup> was also employed as an adjunct in 23 cases. If any

<sup>1</sup> We are indebted particularly to Dr. E. B. McKinley, Director of the School of Tropical Medicine, Puerto Rico, and to Dr. H. C. Clark, Director of the Gorgas Memorial Laboratory, Panama, for furnishing facilities for our work and for aid in procuring material.

<sup>2</sup> Straus, A. H., *Amer. Jour. Pub. Health*, 1928, xviii, 1298.

of the Endo colonies differed from the familiar metallic colonies of the *B. coli* type, several from each plate were picked and plated on standard agar. If the culture appeared pure after growth for twenty-four to forty-eight hours, five colonies were transferred to agar tubes. Inoculation from these was subsequently made into dextrose, lactose and sucrose broth for preliminary identification, tests being also made for indol production and gelatin liquefaction. Mixed cultures were purified by replating. All those strains were preserved for study which, by the tests just mentioned, seemed to possess characteristics of the paratyphoid, typhoid-dysentery or Morgan groups. The majority of those strains that were fished and later discarded were either slow lactose fermenters,<sup>3</sup> various *Proteus* types or an occasional *E. alkaligenes* culture.

Bacteria of the *Salmonella* (paratyphoid) group were isolated twice, in both instances from patients suffering from acute attacks of diarrhea. One of these (868) is culturally of the Aertrycke type, fermenting dextrose, sorbitol, rhamnose, maltose, xylose, arabinose, dulcitol, trehalose and inosite, producing  $H_2S$  and acidifying tartrate medium; not liquefying gelatin, not producing indol, not fermenting lactose, saccharose or salicin. It does not, however, correspond serologically with the Aertrycke (mutton) type and its exact serological relations will be discussed in another place. The other strain (869) is also a free lance strain divergent from the paratyphoid types most commonly met with. It has all the cultural characters above noted for 868 except that it does not attack inosite. Culturally this places it in the Enteritidis-Newport group. It corresponds serologically with the Newport type.

The Morgan bacillus ("Morgan No. 1") was found six times, five times in children suffering from diarrhea of undiagnosed character, once in "suspected typhoid."

The typhoid bacillus was isolated once from a case reported as "enteritis" [64]; once from a case diagnosed "sprue" [32]; once from a case of "suspected milk poisoning" [88].

<sup>3</sup> See, for example, Dudgeon, L. S., and Pulvertaft, R. J. V., Jour. Hyg., 1927-28, xxvi, 285.

Bacteria of the dysentery group were twice found: in one patient with "sprue;" in one diabetic patient without gastrointestinal symptoms. These two "dysentery-like" bacilli were carefully studied with reference to their fermentation abilities and agglutination reactions. The results may be briefly summarized:

PR 134b Enteritis patient. Dextrose, mannitol, rhamnose, maltose, xylose, trehalose and arabinose broth acidified within twenty-four hours; delayed acid production in lactose (five days) and saccharose (fourteen days) broth; no acid production in sorbitol, dulcitol or salicin; non-motile at 22° and 37°; gelatin not liquified; indol not produced; hydrogen sulphide test positive. Agglutinated slightly in 1:100 Flexner bacillus serum; no agglutination at 1:100 in Sonne or Shiga serum.

This bacillus culturally resembles the Sonne bacillus except that it ferments xylose and produces H<sub>2</sub>S; and "B. dispar" of the Lister Institute,<sup>4</sup> except that it does not ferment sorbitol.

PR 136b Hospitalized diabetes patient. Dextrose, mannitol, sorbitol, rhamnose, maltose, trehalose and arabinose broth acidified within twenty-four hours; delayed acid production in sucrose broth; no acid production in lactose, salicin, xylose or dulcitol broth; non-motile at 22° and 37°; gelatin not liquified; indol produced; hydrogen sulphide test delayed positive. Agglutinated slightly, 1:100, with Flexner and with Sonne serum; no agglutination with Shiga serum. This strain is probably to be regarded as belonging to the group of Flexner bacilli. Bacilli of the Flexner type have been reported in Puerto Rico by Costa Mandry and Garrido Morales.<sup>5</sup>

In the Panama Canal Zone and surrounding territory very different conditions from those in Puerto Rico obtained at the time of our visit (January to March, 1931). Although typhoid and dysentery were common in this region during the years when the construction of the canal was first undertaken, they have now practically disappeared. The water supplied to the inhabitants of the Zone and of Panama City is effectively chlorinated, all milk sold is pasteurized, a good sewage disposal system is maintained in the Zone and its environs, and fly-breeding is kept at a

<sup>4</sup> Cf. Koser, et al., Jour. Prev. Med., 1930, iv, 477.

<sup>5</sup> Costa Mandry, O., and Garrido Morales, E., Boletin de la Assoc. Méd. de Puerto Rico, 1931, xxiii, 22.

minimum. In general the conditions for the spread of intestinal infections are no more favorable than in any well-sanitated area in the temperate zone. Relatively few cases of enteritis could be found for examination in spite of the active coöperation of hospital and dispensary physicians.

Thirty-seven stool specimens from 32 individuals, mostly adult patients in the hospitals of the Zone and Panama City, were examined by the methods already outlined. The majority of these persons were suffering from various obscure disorders in which diarrhea was a prominent symptom. This group more closely resembles a group of Chicago hospital patients recently studied by one of us<sup>6</sup> than the group of children ill with "diarrhea and enteritis" observed in Puerto Rico. The results from this group of Panama patients were negative as regards finding any bacilli of the Salmonella or Morgan bacillus types. Slow lactose fermenters were found in about the same proportion as in the Chicago study.<sup>6</sup> Proteus was found once and an organism with the cultural characters of the Sonne dysentery bacillus was found once [56]; the latter did not agglutinate, however, with Sonne bacillus serum. This organism (56 a, b, c) was isolated from a patient with clinical dysentery in the Gorgas Hospital. Dextrose, mannitol, rhamnose, maltose, trehalose and arabinose broth acidified within twenty-four hours; delayed acid production in lactose (four days) and sucrose (fourteen days) broth; no acid production in sorbitol, xylose, dulcitol or salicin; non-motile at 22° and 37°; gelatin not liquified; indol not produced; hydrogen sulphide production delayed (seven days). In spite of the failure of this bacillus to agglutinate with Sonne bacillus serum, the exact cultural correspondence would seem to place it definitely as a member of this group.

Examinations were made in the same way of the stools of 110 Panama school children living in small native villages along the banks of the Chagres river; 107 of these children were, to all appearances, in normal condition, the other three were suffering from mild diarrheal attacks at the time the stool sample was ob-

<sup>6</sup> McBroom, Josephine, Jour. Prev. Med., 1930, iv, 239.

tained. *E. alkaligenes* was found in one of the latter; the other two diarrheal stool samples yielded no organism other than the ordinary *B. coli* type. The 107 specimens of feces from apparently normal Panama children yielded in several instances initially paratyphoid-like bacilli with reaction (dextrose +, lactose -, sucrose -, gelatin -). These organisms produced indol abundantly and did not agglutinate with any available Salmonella serum. The characters enumerated might seem to indicate relationship to the Morgan bacilli, but unlike the latter the fermentation abilities are not limited to the monosaccharides. Some of these bacilli ferment sorbitol, arabinose, etc.; others also ferment salicin; we did not find any biochemical or antigenic agreement among the few strains that were isolated. They may be tentatively placed somewhere between the slow lactose fermenters of the *Bact. coli* group and the Morgan or *Bact. metacoli* types.

#### DISCUSSION

The observations here recorded suggest that in tropical countries such as Puerto Rico where "enteritis" is frequently reported as a cause of death, particularly among children, systematic bacterial examination would show a considerable number of unrecognized typhoid infections, and also many infections with various types of paratyphoid and dysentery bacilli. The relative importance and the sources of these infections are matters for local determination.

In a well-sanitated tropical area, on the other hand, such as the Panama Canal Zone, where enteritis is ordinarily not common, the bacterial flora of hospitalized adults does not differ noticeably from that of a similar population group in a northern city.<sup>6</sup> Even among the children in native villages in the vicinity of the Canal Zone there is little evidence of diarrheal disease, although excreta disposal is primitive and Chagres river water is used for drinking. A rather extensive bacterial examination did not show that the children were harboring any of the well-known intestinal pathogens.

Bacteriologically, it was interesting to find in Puerto Rico a hitherto unreported member of the paratyphoid group. The

Newport strain of Salmonella to which one organism found in Puerto Rico is closely related has previously been reported only from Great Britain and a few localities on the continent of Europe.<sup>7</sup>

Bacteria culturally identical with Sonne and Flexner types of dysentery bacilli were also found in several instances. Although these organisms did not show serological correspondence with the type strains with which they were tested, it does not seem possible to deny them a place in the group of "dysentery bacilli."

The occurrence of the Morgan bacillus in the stools of a number of Puerto Rican children suffering from enteritis, and its apparent absence in healthy persons, may perhaps be regarded as an argument for the causal relationship of this organism to certain intestinal affections. The Morgan bacillus was not found at all in the well-sanitated Canal Zone and its environs.

The fragmentary observations here reported emphasize the desirability of an intensive and prolonged study of tropical enteritis. While the typhoid bacillus and the more familiar varieties of paratyphoid bacilli must doubtless be accorded pathological significance, it is also of value to determine the etiological importance of such organisms as serologically aberrant dysentery and paratyphoid bacilli and alleged pathogens such as the Morgan bacillus and Castellani's *B. columbensis*. The distribution and epidemiological relations of the several types of tropical bacterial "enteritis" deserve investigation.

<sup>7</sup> Lovell, R., Bull. Hyg., 1932, vii, 405.