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REPORT OF THE ROUND TABLE ON THE INTESTINAL MICROBIOTA OF MAN*

This is a report on the contents of a round table discussion on the intestinal microbiota of man presented at the II Central American Congress of Microbiology, held in Panama City, December 1 to 6, 1968. The discussion was sponsored by the Pan American Health Organization, the Institute of Nutrition of Central America and Panama and the Organizing Committee of the Congress.

The program of the Round Table was as follows:

THE INTESTINAL MICROBIOTA OF MAN

December 3, 1968

Organized by: Leonardo J. Mata

INTRODUCTION: Leonardo J. Mata

MICROORGANISMS WITH PATHOGENIC POTENTIAL
Moisés Béhar, Chairman

Intestinal Protozoa with Pathogenic Potential
E. Kotcher

Enterobacteriaceae
Miguel Kourany

Techniques for Isolation of Pathogenic Aerobes and Anaerobes from the Gastrointestinal Tract
Harriette D. Vera

Isolation of Viruses from Feces in a Long Term Study of Diarrhea in Children
Alvaro Dueñas

Pathogenesis of Intestinal Infections
Helmuth Sprinz

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*Prepared for the Eighth Meeting of the PAHO Advisory Committee on Medical Research by Dr. Leonardo J. Mata, Division of Microbiology, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala.

THE INDIGENOUS FLORA

Russell W. Schaedler, Chairman

Experimental Implantation of the Microflora
Russell W. Schaedler

Development of the Fecal Flora in the Breast-Fed Infant
Leonardo J. Mata

Characteristics of the Intestinal Microflora of Man
Enrique de la Cruz

The Relationship of the Bacterial Flora of the Small Intestine to Diarrhea and Malabsorption in Variants of the Blind Loop Syndrome
Franz Goldstein

DISCUSSION

The participants, the floor, and the following invited scientists: Julio I. Colón, Robert Gohd, Cecilia Lizano, Juan J. Urrutia, Arnoldo Ventura, and Richard Wyatt.

In the preparation of this report I have taken the liberty to alter the sequence of presentation of papers and their corresponding discussion, and to refer to certain published and unpublished information not presented in the conference.

INTRODUCTION

Under normal conditions the child is born free of microorganisms. Soon after, however, there is an invasion by numerous species, some of which effectively colonize

vast areas of the body, mainly the skin, certain portions of the gastrointestinal tract, the upper respiratory tract, and part of the lower genitourinary paths (Rosebury, 1962; Marples, 1965).

Studies in gnotobiotic animals have unveiled the biological significance of the intestinal microbiota. It has been shown that the microflora has morphological and physiological effects on the intestinal mucosa (Miyakawa, 1959; Gordon, 1959; Luckey, 1965), determines in part the development of immunological competence (Thorbecke, 1959; Bauer et al., 1963), provides a defense mechanism against infection and other varieties of stress (Dubos et al., 1963), and contributes to the host nutrition by improving food utilization or by synethetising nutrients in situ (Wostmann, 1959; Dubos and Schaedler, 1962; Levenson and Tennant, 1963).

On the other hand, observations in hospitalized patients permitted an evaluation of the significance of the microbiota in the nature and evolution of certain syndromes (Donaldson, 1964). The relationships of the microflora to pathologic processes has been illustrated for diarrhea (Wiejirs and van de Kamer, 1965; Thomson, 1955; Dammin, 1965); malabsorption

(Goldstein et al., 1961; Tabaqchali and Booth, 1967); Whipple's disease (Kok et al., 1964); and auto-immune phenomena (Taylor, 1965; Bregman and Kirshner, 1965).

Although a considerable amount of scientific information has accumulated regarding the relationship of the microbiota with health and disease, much needs to be learned. In the first place, certain components of the microflora can only be efficiently cultivated by applying methods that have become available recently (Hungate, 1950; Schaedler et al., 1965; Moore, 1966, Brewer and Algeier, 1966), and the criteria to characterize the organisms are imperfect or not yet available. Furthermore, a considerable proportion of the total intestinal bacteria has not been isolated nor characterized at all because of their exacting nutritional characteristics and exaggerated susceptibility to oxygen.

Aside from methodological considerations, there is limited information on the microflora of man (Gorbach et al., 1967; Mata et al., 1969a), particularly regarding the development of the microflora of children from preindustrial countries under natural conditions, and of its relation to health and disease. These are fundamental questions, since

the diarreas account for a considerable proportion of the total morbidity, interacting with malnutrition and resulting in one of the leading causes of death in preindustrial regions (Béhar et al., 1958).

DEVELOPMENT OF THE INDIGENOUS INTESTINAL MICROFLORA

The species of microorganisms cultured from the human intestine are typical of this and other animal species, and are not characteristic of the outside environment, that is, they are not generally found saprophytically in nature (Rosebury, 1962). The origin of human intestinal microorganisms is therefore from members of the species, and its acquisition begins immediately after birth.

As indicated in Dr. Schaedler's paper (1968), the indigenous or autochthnous flora is composed of microbial species which have achieved a symbiotic status with the host through a long evolutionary association. The development of the microflora has been studied only in certain animal species (Smith and Crabb, 1961; Schaedler et al., 1965). Using germfree mice, Schaedler (1968) was able to implant certain bacterial species isolated from ordinary mice. Implantation was successful under certain conditions, with

demonstration of the organ specificity and symbiotic nature that characterizes the flora, and of the protective effect against bacterial invaders from the outside.

Ethical and methodological reasons preclude this type of research with humans. The protective effect of the flora has been demonstrated, however, by artifical colonization of the skin of the newborn infant with an inocuous Staphylococcus strain, which interferes with the establishment of the nosocomial pathogenic varieties of Staphylococcus (Shinefield et al., 1963).

The intestinal microbiota of man must, therefore, be studied by a different approach. The development of the fecal microflora of breast-fed Guatemalan Indian children was studied in the village setting, with minimal disturbance of the prevailing conditions as reported in the Conference (Mata and Urrutia, 1968). Meconium and feces collected within three hours after birth were sterile for bacteria. Half of the specimens collected from 3 to 11 hours after birth showed bacteria averaging 10^5 colony forming units (CFU) per gram of wet feces. By 24 hours, all children were colonized with bacteria ranging from 10^{10} to 10^{11} CFU per gram. During the first week of life a varied flora was observed including

lactobacilli, bifidobacteria, non-spore forming Gram-negative anaerobic bacilli (bacteroides), clostridia, and a variety of aerobic cocci and bacilli. This flora varied with time in the same child and also varied among children, but tended to stabilize by the end of the first week, when bifidobacteria became predominant, reaching an average of 10^{11} CFU per gram and representing more than 99% of all culturable bacteria during the period of exclusive breast feeding, Table I. The coliforms were found in lesser concentrations (10^{6} to 10^{10} CFU per gram). Clostridia, bacteroides, lactobacilli and other bacteria were rarely isolated in large numbers after stabilization of the flora.

The intestinal microflora was quite stable during periods of health, but marked changes were observed in children the day of onset of diarrhea, or one or two days after. Alterations consisted in marked proliferation of coliforms (to reach levels of 10^{11} CFU per gram) and relative or absolute decrease of the anaerobic components. The significance of these alterations are being investigated.

With progressive weaning, which in rural Guatemala takes from 2 to 3 years or longer, the flora begins to change.

In the second year of life, for example, bifidobacteria become less numerous than in the first six months of life, although they still predominate. Other anaerobes, mainly the fastidious bacteroides appear more regularly and often in high counts like bifidobacteria. The process of weaning is accompanied by changes in the flora consisting in a progressive proliferation of other groups of anaerobes (like bacteroides) and a relative increase in coliforms which rise from less than 1% to no more than 10% of the total culturable flora, Tables I and II. These changes in flora run parallel to progressive introduction of foods, increased contacts with the contaminated environment, and developing weanling diarrhea. The flora of breast-fed children is "simple" when compared to that of the weanling and adult. The weanling has a transitional flora between that of the breast-fed child and that of the adult. In this, bacteroides is the predominant group with counts of the order of 10^9 to 10^{11} CFU per gram of wet feces, while bifidobacteria are less frequent and numerous, Table II.

THE MICROFLORA AS A DEFENSE MECHANISM

Experiments with animals have demonstrated a protective effect of the microflora against various types of stress including infection. The vulnerability of the germfree animal

to bacterial invasion even with organisms usually of low or no pathonegic potential, has been well documented (Rayniers et al., 1959). The protective effect of the flora has to do with specific microbial actions in situ, with the immunological capacity present under normal conditions and resulting from bacterial stimulation, and other mechanisms. In conventional laboratory animals, certain manipulations that induce changes in the flora are accompanied by increased susceptibility to infection (Dubos and Schaedler, 1959). For instance, treatment with antibiotics is accompanied by marked changes in the relative proportion of bacterial components (Miller et al., 1954; Dineen, 1961; Bohnhoff et al., 1964a; Bonhoff et al., 1964b), rendering the host more susceptible to certain infections and to their associated clinical manifestations.

In vitro and in vivo studies provide a basis to understand the kind of interaction that bacteria show in dynamic systems like the intestinal tract. Dr. de la Cruz (1968) reported his experiments to characterize antagonistic effects between indigenous and pathogenic intestinal bacteria. For the in vitro studies, several species of bacteroides, bifidobacteria (Lactobacillus bifidus) and Shigella flexneri 2a were cultured in a continous flow system. Growth of the various species was

of the order of 10^7 to 10^8 organisms per ml of fluid. When Shigella was introduced into a chemostat containing an active continous flow culture of bifidobacteria plus one of the species of bacteroides, inhibition of the Shigella occurred when the species was Bacteroides fragilis, Table III. The relative numbers of bacteroides and bifidobacteria were basically the same throughout the experiment. Bifidobacteria and B. fragilis also were tested independently for their inhibitory action on Shigella; B. fragilis alone showed an antagonistic effect against dysentery bacilli, although in a lesser degree than that induced by the two species combined (De la Cruz, 1967).

These studies were complemented with in vivo experiments with ligated intestinal loops prepared in the rabbit. A typical tissue reaction and exudate was observed in the loop after inoculation of 10^8 bacilli of Sh. flexneri 2a. Experiments were made consisting in the inoculation of the Shigella with several species of bacteroides and bifidobacteria; proper controls were included. Only B. fragilis diminished the biological effect incited by Shigella. Not only was the tissue response reduced when B. fragilis was inoculated, but also the numbers of dysentery bacilli were reduced 200 fold as compared to the controls.

The in vitro and in vivo studies described show bacterial antagonism. It is possible that antagonistic actions of certain intestinal bacteria are responsible at least in part, for the obvious resistance against Shigella infection observed in the breast-fed infant. Shigella infection has been sporadically demonstrated in breast-fed children in the neonatal period (Mata et al., 1969c). These infections, however, vary in clinical behaviour from those observed in artificially fed children (Haltalin, 1967). That Shigella and other pathogens have opportunities to invade the host is evident from the fecal excretion of pathogens even at an early age (Mata et al., 1967). However, established infection in wholly breast-fed infants is rare, and when it occurs it is usually asymptomatic, and rapidly eliminated. With progressive weaning Shigella infection not only increases abruptly, but the organisms often tend to persist in the gut for weeks or months (Mata et al., 1969b). The factors responsible for resistance are not known. Because the flora of breast-fed differs from that of non breast-fed infants, it seems reasonable to suspect the flora as a possible mechanism of defense in the child. Bacterial inhibitors and antibodies in milk should also be considered.

The significance of the microbiota also becomes evident when it is modified or suppressed by antibiotics. When this occurs, establishment of usually non indigenous microorganisms such as coagulase-positive Staphylococcus, Pseudomonas and Candida albicans, has often been observed.

SIGNIFICANCE OF THE MICROFLORA IN CHRONIC DIARRHEA AND MALABSORPTION

The role of bacteria in chronic diarrhea and malabsorption deserves special consideration. First, because it has been well characterized in the blind loop syndrome as described in Dr. Goldstein's paper (Goldstein et al., 1968). Secondly, because the pathogenesis of malabsorption and diarrhea in this syndrome may be similar to that of tropical sprue and the non-specific diarrheas prevalent in tropical and subtropical regions. Also, because antibiotics have been shown to have beneficial effects in patients with absorptive disorders (Wirts and Goldstein, 1963; Sheehy and Pérez-Santiago, 1961).

The blind loop syndrome in its broadest definition includes all conditions in which there is stasis of small bowel contents, bacterial overgrowth and resultant diarrhea and malabsorption. Therefore, it includes not only the classical intestinal blind

loops, anastomosis and strictures, but the postgastrectomy steatorrhea, intestinal scleroderma and intestinal diverticulosis.

Most investigators agree that in the duodenum and proximal small intestine, microorganisms are not common and, when found, their numbers are negligible, while colonization is prolific in the large intestine. Goldstein and co-workers (1968) using special bacteriologic techniques showed that aspirates from the proximal small intestine had no bacteria or their numbers were negligible in about half the normal persons studied. In the other half, the number of anaerobes was not greater than that of aerobes, and the total bacterial counts averaged $10^{3.6\pm 1.4}$ CFU per ml. The expected normal maximum value would be of the order of 10^6 CFU per ml. On the other hand, aspirates from patients with small bowel disease showed, in general, excessive growth exceeding the above figure, as shown in Table IV for patients with diverticulosis. Suppresion of the abnormal bacterial populations in the jejunum by antibiotics was generally accompanied by an improvement of absorption and relief of diarrhea. Absorption of Vitamin B_{12}, fat, and xylose returned to normal. Patients began to gain weight and malnutrition was corrected. Persons treated in this way

were under surveillance for years without remissions. Ocassionally, relapses occurred and were successfully treated with additional antibiotic therapy.

The deleterious effects of bacterial overgrowth in sites like the upper small intestine on absorptive phenomena has been confirmed in experimental animals. In rats, to mention an example, enteric microorganisms impair absorption of vitamin B_{12} and lipids (Donaldson, 1967). One way bacteria interferes with absorption seems to be by hydrolysis of bile salt conjugates or dehydroxylation of the bile salt molecule interfering with the formation of micelles necessary for lipid absorption. Bacteria may also compete with the host for a particular nutrient (Goldstein et al., 1965; Goldstein et al., 1968). Several products, mainly organic acids, are liberated as a result of bacterial metabolism. These may be a source of irritation to the intestine (Donaldson, 1964).

MICROORGANISMS WITH PATHOGENIC POTENTIAL

Under normal conditions, intestinal microorganisms are in a balance with the host through a dynamic metabolic interaction (Rosebury, 1962). These microorganisms constitute the "Indigenous microbiota", characteristic for a host in a

given environment. The indigenous microbiota is formed by microorganisms typically commensal or saprophytic, some having symbiotic functions. In addition, there are pathogenic microorganisms that invade the intestinal tract and become established without causing clinical disease, but that are not usually considered indigenous. These include protozoa (Entamoeba histolytica, Dietamoeba fragilis, Giardia lamblia), certain enteric bacteria (Shigella, Salmonella) and viruses (entero- and adenoviruses). These microorganisms will be arbitrarily labeled "undesirable". The frequency with which they are found in the intestinal tract depends on certain factors such as personal hygiene, environmental sanitation, and state of nutrition. In industrial countries they usually are sporadic or of low prevalence; in tropical and subtropical preindustrial areas, rates of infection of undesirable species are very high.

Dr. Kotcher (1968) reviewed the intestinal protozoa from the standpoint of their pathogenic capacities. He concluded that E. histolytica, D. fragilis, G. lamblia, Balantidium coli, and Isospora belli and I. hominis are definitely associated with diarrheal disease and other gastrointestinal disturbances. Also, Dr. Kourany (1968) indicated that bacteria such as

Shigella, enteropathogenic Escherichia coli, Salmonella and Arizona, have been consistently incriminated as etiologic agents of diarrheal disease while other agents like Citrobacter and Edwardsiella tarda have probable pathogenic potential.

Studies in Central America (Lizano and de Abate, 1953; Brooke et al., 1963; Mata et al., 1965; Kotcher et al., 1967; INCAP, 1969) have indicated a high prevalence of Entamoeba histolytica and other protozoa in the general population, as well as of enteric pathogenic bacteria, Table V. Frequently, children are infected with Giardia and other protozoa, for prolonged periods (INCAP unpublished information). Also, infections with Shigella, thought to be transient, often tend to persist for weeks or months, as does Salmonella, sometimes without overt clinical manifestations (Mata et al., 1969b). Persistance of pathogenic protozoa and bacteria in the intestinal tract, therefore, places them in the position of being almost indigenous.

The situation of viruses in this regard is similar, even though the pathogenic nature of viruses is more easily defined because replication implies a parasitic existence and destruction of the cell, and secondly, because enteric

viruses (and other viruses excreted in feces) are often incriminated in the causation of a wide range of symptoms and illnesses. Fecal virus excretion by children of preindustrial regions is remarkably high, as shown in a long term prospective study in Colombia reported by Dr. Dueñas (Dueñas and Clemmer, 1968). He described an overall prevalence of enteroviruses in the child population of around 60%, Table V. Viral infections in general are short lived, but a considerable number of infections with entero- and adenoviruses last several weeks (INCAP unpublished information). Under these circumstances, it is tempting to talk about a "normal" enteric viral flora as has already been suggested (Gelfand et al., 1957).

The persistence of pathogenic protozoa and bacteria, and of viruses, without associated clinical manifestations, in the intestine of children living in poor environmental conditions, would permit one to classify these organisms as indigenous. On the other hand, the exaggerated prevalence of these agents in the intestinal tract increases the incidence of diarrheal disease and other gastrointestinal problems. Numerous clinical and field studies showed a correlation of these agents with diarrhea, as exemplified

in Dr. Colon's discussion (1968). For the reasons just outlined, the term "undesirable" has been applied to these groups of highly prevalent microorganisms.

PATHOGENESIS OF INTESTINAL INFECTION

In the germ-free state as in the foetus *in utero*, the intestinal mucosa does not show the characteristic "physiologic inflammation" normally found in the post-natal condition (Sprinz, 1962). The physiologic inflammation is stimulated by the presence and action of indigenous microorganisms. As reviewed by Dr. Sprinz (1968), the intestinal mucosa is normally in a state of continuous and rapid renewal. The phenomenon manifests itself morphologically by mitotic figures in the intestinal epithelial chief cells in the generative compartment of the crypts of Lieberkühn, by the movement of these immature cells to the crypt villus junction where maturation is completed, and by the motion of fully functioning cells towards the villus crest where eventual extrusion takes place. Globet cells behave similarly, but enterochrome and Paneth cells do not migrate (Abrams *et al.*, 1963). The process of renewal described above for the epithelial lining, has its counterpart in the lamina propria

which has its own type of cell turnover. Both are stimulated by the intestinal microbiota. The mucosa is then in a stimulated state ready to react to additional stress, such as an infection by one of the undesirable agents or by members of the indigenous flora under certain circumstances. This added stress alters the dynamic equilibrium and exaggerates or distorts the normal pattern of cell turnover. The responses can be of the following categories: (a) increased epithelial cell turnover and cell extrusions; (b) discharge of mucus and altered globet cell population; (c) degenerative changes of intestinal chief cells affecting absorption and secretion, and (d) inflammatory cellular and vascular reaction of the lamina propria with its implication in terms of IgA production. These responses are conditioned by several factors of which the most important ones are: (a) host nutrition and capacity of the tissue to react; (b) the nature of the injurious agent (pathogenicity, virulence) and the intensity of the stimulus (dose) and (c) the anatomical site and organization of the tissue (motility, tonus, absorption, secretion).

In addition to the normal physiological inflammation already mentioned with participation of the indigenous microbiota, four major types of host parasite relationship may be recognized, exemplified by the cholera vibrio, the

intestinal spirochetes,, the dysentery bacilli, and the salmonellae. In the first case, with vibrio organisms there is no invasion of the tissues and the pathogenesis is mediated through the action of absorbed toxins (Gordon et al., 1966). In the second type (Harland and Lee, 1967), infection is characterized by partial penetration of spirochetes in the intestinal epithelial cells (this type has no clinical significance for man). In the third category, dysentery bacilli invade the epithelial cells not only of the intestine but of the stomach as well, and multiply within them, protected from some of the host defense mechanisms. Although in acute shigellosis there is proliferation of bacteria in the lamina propria, the number of bacilli in the submucosa are less, and systematic invasion seems to be rare (Kent et al., 1967).

In the fourth type, salmonella readily cross the epithelial lining, showing less preference for intra-epithelial multiplication and they therefore cause less damage to the intestinal covering (Takeuchi and Sprinz, 1967). The proliferation of Salmonella is accompanied by the production of factors which are chemostatic for neutrophils and monocytes. This determines in part the nature of the inflammatory response

that develops. The pathogenesis of intestinal infections is varied because of the complexity of microbial actions and of host mechanisms responding to the challenge, as well as because of the variety of existing pathogenic agents.

METHODS OF STUDY OF THE INTESTINAL MICROFLORA

Interest and knowledge on the intestinal microflora was restricted until recently to the aerobic components; technical problems encountered in the study of anaerobes was one of the reasons for this situation. Also, the significance of certain enterobacteriaceae (e.g. Shigella, Salmonella) in the etiology of diarrheal diseases led to a concentration of efforts to study that family. Technological advances in gnotobiology, in animal nutrition and animal physiology, and the discovery of antibiotics, as well as the advances in bacteriological techniques have permitted the study of the intestinal flora in toto.

In this conference, a review of the available culture media for the study of enteric aerobic and anaerobic pathogens was made by Dr. Vera (1968). Among the aerobes emphasis was placed on Salmonella, Shigella, Pseudomonas, Proteus, Vibrio, Neisseria, Streptococcus, and Staphylococcus, as well as on

fungi. Techniques and culture media were also reviewed for anaerobic bacteria. Inoculation of thioglycollate medium and of blood agar plates with discs containing 30 mcg of Kanamycin or 10 units of Bacitracin was recommended.

Several papers presented in the conference indicated the use of special techniques to culture the whole intestinal flora. These were the methods of Hungate (1950), Schaedler et al., (1965), and Brewer and Algeier (1966). The latter is among the best systems to obtain anaerobiosis and has great potentialities for work with humans. Most techniques still involve the undesirable exposure to air of the specimens to be cultured. The success to apply the Hungate system to the culture of aspirates from small intestine was indicated (Goldstein et al., 1968). This and other methodologies (Moore, 1966; Lee et al., 1968) are promising in the case of human studies.

SUMMARY

This paper summarizes a conference dealing with a variety of aspects of the intestinal microbiota. The classical approach has been to focus only on the non-pathogenic indigenous microorganisms. In the conference, consideration was also given to the "undesirable" agents because of their omnipresence

in the intestinal tract of children of preindustrial areas, constituting virtually indigenous components of the microbiota. References were made to the methodology necessary to study the flora. A report was made on the development of the fecal flora in children studied under natural conditions, and on the composition of the flora of breast-fed children, weanlings and adults. The importance of the flora as a defense mechanism was illustrated with experiments in vitro and in vivo. The pathogenic capacities of certain protozoa, bacteria and viruses, and the pathogenesis of intestinal infections, particularly by agents that cause diarrhea, were reviewed. The abnormal colonization of the proximal small intestine and its relation to malabsorption and chronic diarrhea was also illustrated with examples of a specific syndrome.

It became evident that the study and understanding of the role of the intestinal microbiota in health and disease is necessary in areas where diarrheas are still one of the leading causes of disability and death. The problem is evidently complicated because the microbiota is a complex "unit" formed by a great variety of microorganisms (many not yet isolated or characterized), in a dynamic interaction with each other and with the host, and subjected to influences from the host and environment.

Unquestionably, more research is needed if an adequate understanding of the functions of the microbiota is to be obtained. Research should be directed to the following aspects: (a) a better characterization of the indigenous species. This should contemplate the use (or development) of new methodology and the study of the predominant organisms by physiologic, immunological, and other methods; (b) study of the microbiota in samples obtained from several segments of the gastrointestinal tract. This should be in normal persons as well as in individuals with acute and chronic non-specific diarrhea, malabsorption and protein-calorie malnutrition; (c) study of the interrelations between indigenous and undesirable agents; and (d) study of host-parasite relationships at the mucosa level, in human biopsies and autopsy material, and in experimental animals. The knowledge that can be gained from such studies will provide a basis for better control and prevention of diarrhea, malabsorption and other intestinal disorders.

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Table I

FECAL BACTERIAL FLORA OF BREAST-FED CHILDREN STUDIED IN THE FIRST YEAR OF LIFE *

Santa María Cauqué, Guatemala, 1967-1969

BACTERIAL GROUP	AGE IN WEEKS 1-4	9-12	17-20	25-28	33-36	41-44	49-52
Bifidobacteria	11.4**	10.7	11.5	11.3	11.2	11.1	11.1
Enterobacteriaceae	5.7	7.4	8.9	9.0	9.7	9.0	9.2
ANAEROBES	11.5	11.6	11.7	11.4	11.6	11.1	11.1
AEROBES	7.6	8.4	9.0	9.4	9.7	9.4	9.4
Ratio ANAEROBES to AEROBES	7900/1	1600/1	500/1	100/1	80/1	50/1	50/1
% ANAEROBES IN TOTAL FLORA	>99.9	>99.9	99.8	99.0	98.8	98	98

(*) Adapted from Mata and Urrutia (1968)

(**) Average $\log_{10}$ of number of colony forming units per gram of wet feces.

Table II

FECAL BACTERIAL FLORA IN HEALTHY PERSONS*
SANTA MARIA CAUQUE, GUATEMALA

Bacterial group	Breast-fed children, 2 to 4 months old	Weanlings 2 to 3 years old	Adults 23 to 37 years old
Bifidobacteria	11.4** (19/19)	10.6 (12/12)	9.4 (9/12)
Bacteroides	10.7 (3/19)	9.2 (3/12)	10.3 (12/12)
Clostridia	10.0 (3/19)	10.0 (2/12)	9.3 (7/12)
Veillonellae	9.8 (16/19)	9.6 (7/12)	9.2 (6/12)
Enterobacteriaceae	8.3 (17/19)	8.0 (12/12)	8.7 (12/12)
ANAEROBES	11.5±0.5***	11.0±0.4	10.5±0.7
AEROBES	8.3±1.1	8.0±1.0	8.8±0.6

(*) Adapted from Mata et al., 1969

(**) Averages of log10 number of colony-forming units per gram of wet feces. Fractions indicate the cases bacterial group found in the total number of cases cultured.

(***) Averages of log10 ± one standard deviation.

Table III

EFFECT OF CONTINUOUS FLOW CULTURES OF BIFIDOBACTERIA AND BACTEROIDES ON THE GROWTH OF *SHIGELLA FLEXNERI 2a*.*

Bacteria in flow culture	Number of Shigella organisms per ml.						
	0 hr.	1 hr.	5 hr.	7 hr.	12 hr.	24 hr.	48 hr.
Shigella, alone	10^2	10^2	10^5	10^7	10^8	10^8	10^8
Shigella + bifidobacteria							
+ *B. vulgatus*	10^2	10^2	10^4	10^6	10^6	10^7	10^8
+ *B. varius*	10^2	10^2	10^4	10^7	10^7	10^8	10^8
+ *B. fragilis*	10^2	10^2	10^3	10^4	10^6	10^6	10^6

(*) Adapted from De la Cruz (1967)

Table IV

BACTERIA IN ASPIRATES FROM THE SMALL INTESTINE
OF PATIENTS WITH DIVERTICULOSIS*

Patient	Age (years and sex)		Clinical State	Highest daily fat excretion gm/day	Colony-forming units of predominant organism/ml
1	68	M	Severe diarrhea Malabsorption Weight loss	18.2	$>10^9$ Escherichia coli
2	76	M	Severe diarrhea Malabsorption Weight loss	37.9	1.2×10^9 E. coli
3	61	M	Severe diarrhea Steatorrhea Weight loss	10.9	6×10^7 E. coli
4	43	F	Mild diarrhea	4.7	no growth
5	63	M	Asymptomatic	5.8	1.2×10^7 E. coli
6	74	F	Asymptomatic	5.3	1.6×10^7 E. coli
7	71	F	Asymptomatic	1.5	3.7×10^4 E. coli

(*) Adapted from Goldstein et al., (1968)

Table V

FECAL EXCRETION OF UNDESIRABLE AGENTS BY CHILDREN OF PREINDUSTRIAL COUNTRIES

Agent	Country	Age in years	Percent Prevalence	Source
Entamoeba histolytica	Costa Rica	5-9	28.5	Kotcher *et al.*, 1967
Giardia lamblia	El Salvador	1-4	29.0	INCAP, 1969
Shigellae	Guatemala	5-9	7.2	Mata *et al.*, 1965
Enteroviruses	Colombia	0-2	60.0	Dueñas and Clemmer, 1968