MMI MEDICAL MICROBIOLOGY AND IMMUNOLOGY

ɔurnal publishes original papers in the ɔf virology, bacteriology, immunology and miology. Pathogenetic mechanisms as ·lying causes of origin, treatment and ıylaxis of infectious diseases constitute ɔre of its contents, whereas its nitment to problems of basic research in biology is merely secondary. Manuscripts and correspondence concerning editorial matters may be submitted to any of the editors, preferably a specialist in the field to which the subject of the article belongs. For addresses see bac of the issue.

Subscription Information

Volume 173 (6 issues) will appear in 1984. The price of the volume is DM 308.00 or approx. US $ 122.00. Prices for back volumes are available on request. Correspondence concerning subscriptions should be addressed to the publisher.

North America. Annual subscription rate: Approx. US $ 128.00 (single issue price approx. US $ 26.00) including carriage charges. Subscriptions are entered with prepayment only. Orders should be addressed to:
Springer-Verlag New York Inc.
Service Center Secaucus
44 Hartz Way
Secaucus, NJ 07094, USA,
Tel. (2 01) 3 48-40 33, Telex 0 023-125 994.

All other countries: Annual subscription rate: DM 308.00 plus carriage charges. Airmail delivery on request only. For *Japan,* carriage charges (Surface Airmail lifted) are DM 44.60, for *India* DM 27.90. Single issue price: DM 61.60 plus carriage charges. Orders can either be placed with your bookdealer or sent directly to:
Springer-Verlag
Heidelberger Platz 3
D-1000 Berlin 33
Tel. (0) 30/82 07-1, Telex 1-83 319.

Changes of address: Allow six weeks for all changes to become effective. All communications should include both old and new addresses (with Postal Codes) and should be accompanied by a mailing label from a recent issue.

Microform: Microform editions are available from: University Microfilms International, 300 N. Zeeb Road, Ann Arbor, MI 48106, USA

Production
Springer-Verlag
Journal Production Department II
Postfach 10 52 80
D-6900 Heidelberg 1
Federal Republic of Germany
Tel. (0) 62 21/4 87-3 41, Telex 4-61 723.

Responsible for advertisements
Springer-Verlag
E. Lückermann
Kurfürstendamm 237
D-1000 Berlin 15
Tel. (0) 30/8 82 10 31, Telex 1-85 411.

Printers
Composed by
Conscript, Inh. B. Steiner, Heidelberg
Printed by Konrad Triltsch, Graphischer Betrieb, Würzburg

Med Microbiol Immunol
(1984) 173:113–125

Medical Microbiology and Immunology

Comparison of antitumor activity of *Lactobacillus casei* with other bacterial immunopotentiators

N. Yasutake,* M. Ohwaki, T. Yokokura and M. Mutai

Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi-shi, Tokyo 186, Japan

Abstract. Antitumor activity of *Lactobacillus casei* YIT 9018 (LC9018) was demonstrated by intralesional (i. l.) or intravenous (i. v.) administration into tumor-bearing mice which were inoculated with methylcholanthrene-induced fibrosarcoma (Meth A) or Kirsten murine sarcoma virus-transformed tumor (K234) cells. Its activity was significantly superior to the activity of two other species of lactobacilli but was nearly the same as that of *Corynebacterium parvum* or *Mycobacterium bovis* Bacille Calmette-Guérin (BCG). I. l. or i. v. administration of LC9018 into the tumor bearers caused local transient swelling or hepatosplenomegaly but did not cause other pronounced lesions. There was no significant difference in the degree of hepatosplenomegaly in LC9018 and that in other immunopotentiators. In mice whose tumors had regressed as a result of administration of LC9018 or the other immunopotentiators, the phytohemagglutinin P (PHA-P) response of the spleen cells was less than that of mice whose tumors progressed, and approached the normal level. The PHA-P response of popliteal lymph node cells proximal to the tumor lesion was fairly low compared with the splenic PHA-P response and there was no difference between the lymphocytes from mice whose tumors had regressed or progressed. Adjuvant activity of LC9018 in inducing tumor immunity was demonstrated by administering a mixture of LC9018 and Meth A cells to mice. This adjuvant activity was of the same efficiency as that of *C. parvum* and BCG. The presence of the antitumor activity of LC9018 in cell wall components was deduced from fact that removal of its cell wall by endo-N-acetylmuramidase (M-1 enzyme) abolished the activity. The possible availability of LC9018 for immunotherapy of tumors is discussed.

* Corresponding author

NOTICE: This material may be protected by copyright law (Title 17, United States Code).

GASTROENTEROLOGY 1984;86:174–93

PROGRESS ARTICLE

Intestinal Flora in Health and Disease

GARY L. SIMON and SHERWOOD L. GORBACH
Division of Infectious Diseases, The George Washington University Medical Center, Washington, D. C., and the Division of Infectious Diseases, Tufts New England Medical Center, Boston, Massachusetts

The Normal Microflora

The bacterial inhabitants of the human gastrointestinal tract constitute an enormously complex ecosystem that includes both aerobic and anaerobic microorganisms. There are more than 400 bacterial species in the colonic flora of a single individual (1). Detailed microbiologic analysis of such a heterogenous mixture is a monumental task; it has been estimated that complete bacteriologic characterization of a single fecal specimen may take up to 1 yr to complete. Fortunately, consideration of only the most representative microorganisms provides a reasonable outline of the gastrointestinal microflora (Table 1).

Bacteria from the oral cavity are washed with saliva into the stomach where the vast majority are destroyed by gastric acid (2). The microflora of the stomach is predominantly gram-positive and aerobic. The bacterial concentration usually is $< 10^3$ colony forming units/ml (CFU/ml), and the most commonly isolated species are streptococci, staphylococci, lactobacilli, and various fungi (3,4). Oral anaerobes such as *Peptostreptococcus*, *Fusobacterium*, and *Bacteroides* species may be present in low numbers, but coliforms, *Clostridium*, and *Bacteroides* are distinctly uncommon.

The small intestine constitutes a zone of transition between the sparsely populated stomach and the luxuriant bacterial flora of the colon. The microflora of the proximal small bowel is similar to that of the stomach. The bacterial concentration is 10^3–10^4 CFU/ml. The predominant species are aerobic and gram-positive, although coliform and anaerobic bacteria can be frequently isolated in low concentrations. In the distal ileum, gram-negative bacteria begin to outnumber gram-positive organisms. Coliforms are consistently present, and anaerobic bacteria, such as *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, and *Clostridium*, are found in substantial concentrations (2–7).

Distal to the ileocecal sphincter, bacterial concentrations increase sharply. Within the colon the bacterial concentration is 10^{11}–10^{12} CFU/ml; nearly one-third of the fecal dry weight consists of viable bacteria (1,8). Anaerobic bacteria outnumber aerobes by a factor of 10^2–10^4. The predominant isolates are *Bacteroides*, *Bifidobacterium*, and *Eubacterium* (1–4,9–11). Anaerobic gram-positive cocci (peptococci and peptostreptococci), clostridia, enterococci, and various species of enterobacteriaceae are also common.

Newborn Infants

Colonization of the gastrointestinal tract of newborn infants occurs within a few days of birth (12). The type of delivery, dietary constituents, and gestational age may influence the colonization pattern of anaerobic bacteria. Long and Swenson (13) found that within 4–6 days nearly all full term, formula-fed, vaginally delivered infants were colonized with anaerobic bacteria, and of these, 61% harbored *B. fragilis*. In contrast, anaerobes were present in 59% and *B. fragilis* in only 9% of infants delivered by cesarean section, suggesting that significant contamination occurred during passage through the birth canal. Both prematurity and breast feeding reduced the likelihood of isolating anaerobic species. Colonization of the small bowel occurs perorally. In newborn infants with congenital small bowel obstruction, a fecal-type flora is found immediately proximal to the site of obstruction, and the distal bowel remains sterile (14).

Techniques for Studying Small Intestinal Flora

The relative inaccessibility of all but the most proximal and distal portions of the gastrointestinal tract has promoted the use of a variety of specialized

Received December 3, 1982. Accepted August 10, 1983.
Address requests for reprints to: G. L. Simon, M.D., Division of Infectious Diseases, The George Washington University Medical Center, 2150 Pennsylvania Avenue, NW, Washington, D.C. 20037.

0016-5085/84/$3.00

Danish group. The Finns also consumed more dietary fiber. Whether the higher intake of dairy products and higher counts of lactobacilli, or the greater intake of fiber, provides a protective effect remains speculative.

In a laboratory model, the effect of feeding lactobacilli was studied in rats consuming either a grain or beef diet (257). When *L. acidophilus* was added to the diet, there was little change in the metabolic activity of fecal bacterial enzymes in grain-fed animals; however, a significant reduction in β-glucuronidase, azoreductase, and nitroreductase activity was noted in rats consuming a beef diet.

These results suggested that feeding lactobacilli might provide some protection from ingested carcinogens. In a subsequent study, beef-fed rats were given DMH to induce colon tumors, along with lactobacilli dietary supplements (258). There was significant reduction in colon cancers at the 20-wk observation time, but not at 36 wk. These findings suggest that dietary lactobacilli increase the latency or induction time for colon cancers, probably by influencing the metabolic activity of the colonic flora.

Intestinal Microflora and the Enterohepatic Circulation of the Sex Steroid Hormone

The metabolism of the sex steroid hormones involves an enterohepatic circulation that is dependent upon a biologically active microflora. These hormones undergo a cycle of biliary excretion, bacterial deconjugation, and intestinal reabsorption similar to that of the bile acids. Approximately 60% of circulating estrogens are conjugated in the form of glucuronides or sulfates, and are excreted in the bile (259–264). Deconjugation, the prerequisite step for mucosal cell reabsorption, is catalyzed by the bacterial enzymes, β-glucuronidase, and sulfatase. Deconjugation is nearly complete. Indeed, the small amount of estrogens excreted in the feces is 97% in the deconjugated form, although virtually all of the estrogens in bile are conjugated (265).

Intravenous administration of radiolabeled estriol has demonstrated a two-step conjugation process in the liver (260,264). Initially, glucuronide is formed. This is subsequently converted to estriol-3-sulfate-16-glucuronide and excreted in the bile. In the bowel, bacterial β-glucuronidase and sulfatase hydrolyze this doubly conjugated moiety to free estriol, most of which is reabsorbed by the mucosal cell. In the mucosal cell, free estriol is reconjugated to estriol-16-glucuronide (266). This compound returns to the liver, where it is further conjugated to form estriol-3-sulfate-16-glucuronide, which completes the enterohepatic cycle (267). The mucosal cell can also reconjugate to estriol-3-glucuronide, a compound that is resistant to further conjugation. This moiety does not undergo an enterohepatic circulation, and it is fated for rapid excretion in the urine. The presence of estriol-3-glucuronide can be used as an indicator of estrogen reabsorption in the lower gastrointestinal tract since the synthesis of this compound occurs only in mucosal cells of the colon.

Dietary consideration appears to have an effect on the excretion of estrogens. Goldin et al. (268) found that among premenopausal vegetarian women the fecal excretion of estrogen was increased threefold when compared with omnivores. Urinary estriol-3-glucuronide was also reduced in vegetarians. These findings suggest that the enterohepatic circulation of estrogens is reduced in vegetarian women.

Antibiotics have a profound effect on the enterohepatic circulation of estrogen. Studies have shown that there is a decreased urinary estriol concentration after oral administration of ampicillin, penicillin, or neomycin (269–271). The last antibiotic is of particular interest because it is poorly absorbed after oral administration, indicating that the observed effect is due to changes in the makeup of the intestinal flora and not to any systemic properties of the drug. When antibiotics are given, the urinary levels of total conjugated estriol and estriol-16-glucuronide were reduced, along with a 60-fold increase in the fecal excretion of conjugated estrogens and a threefold increase in unconjugated moieties (272–274).

Tikkanen et al. (275) studied the effect of ampicillin on estriol metabolism and noted that 90% of the observed reduction after antibiotic administration could be accounted for by a decrease in the urinary excretion of the 3-glucuronide. These findings confirmed the gastrointestinal origin of this effect because the synthesis of this conjugate occurs only in the intestinal mucosal cells. These results are most consistent with the postulate that the effect of antibiotics on the excretion of estriol is through suppression of the microflora with a consequent decrease in the bacterially mediated deconjugation reaction.

The metabolism of progesterone is also subject to alterations after the administration of antibiotics (272,276,277). Adlercreutz et al. (272) found that the urinary concentration of glucuronide-conjugated metabolites was markedly decreased by the second day of ampicillin administration. These values had risen to near normal levels, however, by the third day.

A recent study of androgen metabolism in the beagle dog has revealed similar findings (278). On the second day of ampicillin administration, a sharp increase in fecal androgens was noted; this increase began to normalize by the following day. The in-

INFECTION AND IMMUNITY, Feb. 1978, p. 704–710
0019-9567/78/0019-0704$02.00/0

Vol. 19, No. 2
Printed in U.S.A.

Inhibition of *Neisseria gonorrhoeae* by Aerobic and Facultatively Anaerobic Components of the Endocervical Flora: Evidence for a Protective Effect Against Infection

JEAN H. SAIGH, CHRISTINE C. SANDERS,* AND W. EUGENE SANDERS, JR.
Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178

Received for publication 19 August 1977

The ability of aerobic and facultatively anaerobic endocervical flora to inhibit the growth of *Neisseria gonorrhoeae* in vitro was assayed. Factors influencing the occurrence of inhibitory components of the flora in vivo were evaluated. Endocervical swabs were obtained from 229 women at a local venereal disease clinic. Endocervical flora and *N. gonorrhoeae* were isolated and identified, and the ability of the flora to inhibit the growth of *N. gonorrhoeae* was determined by an agar overlay assay. Results revealed the most active inhibitors to be streptococci, staphylococci, and lactobacilli, in that order. Among only those women harboring inhibitory endocervical flora, inhibitory lactobacilli were recovered from fewer women infected with *N. gonorrhoeae* than uninfected women ($P < 0.05$). Among women having contact with an infected partner, those who subsequently developed gonorrhea were less likely to have inhibitory lactobacilli than those who did not become infected ($P < 0.05$). No other significant differences in the composition of the inhibitory flora were noted between infected and uninfected women. During the 2 weeks following menses, recovery of inhibitory lactobacilli on culture was highest, whereas recovery of *N. gonorrhoeae* was lowest. These observations suggest that the presence of certain lactobacilli may reduce risk of acquisition of *N. gonorrhoeae* following exposure to infected partners and that the potential protective effect may be greatest during the 2 weeks after menses.

Despite the development of effective chemotherapeutic agents and recent intense efforts to control gonorrhea, it has become one of the most prevalent of bacterial infections. Very little is known about factors which influence acquisition of *Neisseria gonorrhoeae* by females. Various factors, such as method of contraception (2, 8, 14) and host immunity (4, 6, 7, 21) have been hypothesized to play a role in susceptibility or resistance to gonorrhea. In addition, it is possible that fluctuations in the composition of the endocervical flora associated with the menstrual cycle may influence acquisition rates.

Many investigators have studied bacterial antagonisms occurring between the normal flora and potential human pathogens in vitro. Studies at other body sites have suggested that antagonistic components of the indigenous flora may play a role in resistance to gram-negative bacillary colonization of the pharynx (32), group A streptococcal colonization of the throat (9, 29), and staphylococcal infections in the newborn (31). To date, however, the possible role of the endocervical flora in resistance to gonococcal infections has not been extensively investigated. Therefore, the present study was designed to (i) determine the ability of aerobic and facultatively anaerobic components of the endocervical flora to antagonize the growth of *N. gonorrhoeae* in vitro and (ii) examine various factors which might influence the occurrence of these organisms in vivo.

MATERIALS AND METHODS

Study design. Endocervical swabs were taken from 229 women reporting to a local "free" clinic for gynecological reasons. Components of the endocervical flora were isolated in pure culture and tested for inhibitory activity against *N. gonorrhoeae* by an agar overlay technique. Cultures for *N. gonorrhoeae* were performed on Thayer-Martin medium. To evaluate host factors that might influence the presence or absence of inhibitory endocervical flora and to minimize possible bias, pertinent history was obtained by only one investigator (J.H.S.). For data analysis, the women were subgrouped according to three parameters: (i) presence or absence of *N. gonorrhoeae* in the endocervix, (ii) week during the menstrual cycle at which the endocervical culture was obtained, and (iii) use of oral contraceptives. Data relating to the menstrual cycle were not available for 22 women. No pregnant women were included in the study population. All tests of statistical significance were performed with chi-square analysis for a 2×2 contingency table with Yates correction factor.

Endocervical cultures. Endocervical cultures

Interactions Among Lactobacilli and Man[1]

M. L. SPECK
Department of Food Science
North Carolina State University
Raleigh 27607

ABSTRACT

Lactobacilli and other lactic acid bacteria have been involved intimately with man for centuries. The associations have involved manufacture of various human foods as well as performance of various beneficial interactions in different parts of the human body. Renewed interests in the role of the intestinal microflora again have focused on the intestinal lactobacilli, particularly *Lactobacillus acidophilus*.

INTRODUCTION

Microorganisms have been involved for centuries in the manufacture of dairy foods consumed by humans. Nondairy foods also have been manufactured by the same types of microorganisms. Many of the interactions of the beneficial types of microorganisms, especially the lactic acid bacteria, have been clarified only in recent years. Nevertheless, these microorganisms have been considered real benefactors of man in his quest for food. It should not be surprising that more recent research indicates interactions between the lactic acid bacteria and man which may explain even greater human benefits than previously had been suspected.

CUSTOMARY USES OF STARTER MICROORGANISMS

Natural food which have been bioconverted by microorganisms add a wide variety to the menu. Metabolic products of starters produce a number of flavors different from those in the original food. Textures of the altered foods also are different from the beginning product. Also, as in cheese, nutrients can be concentrated so that less space is needed for their transportation and storage.

Probably man's first use of starters was motivated by his need to preserve foods. Before conveniences such as refrigeration, sterilization by heat, and preservative chemicals, metabolic activities of microorganisms growing in natural foods permitted man to preserve foods for extended times. The preservation of milk by starters led to the development of modern industries where cultured buttermilk, yogurt and cheeses have become prime items of our diet. However, we may overlook the fact that many other foods have been preserved similarly. Vegetables can be pickled by the use of microorganisms, yielding foods such as sauerkraut and pickles for man and silage for animals. The juices of fruits can be converted into wines and some fruits yield one of the best preservatives that man has ever known, vinegar. Microorganisms can permit the preservation of meat in the form of various types of sausages. The development of sourdough bread, aside from its gourmet qualities, probably enables certain cereals to survive attacks of different microorganisms that would shorten the shelf life of bread. Thus, for many years, man has been dependent on microorganisms to help him preserve his foods. He has been in intimate contact with the viable microorganisms as well as their products of metabolism and growth.

PROPERTIES OF CULTURES THAT FAVOR FOOD PRESERVATION

Acids produced by starter bacteria undoubtedly constitute a most important food preservative. Acids differ in their ability to inhibit microbial growth (2). Even though lactic acid is the predominant acid produced by most starter cultures, this acid is not nearly as effective in inhibiting other microorganisms as is acetic acid. Although acetic acid is produced in small concentrations by some component strains of starters, it is very effective in the inhibitory actions of *Streptococcus diacetilactis* (20) and *Leuconostoc citrovorum* (25). Certain of the lactobacilli produce sufficient hydrogen peroxide to be inhibitory to various microorganisms

Received July 14, 1975.

[1]Sponsored in part by funds from the National Dairy Council.

region of the intestinal tract early in life, multiply to high levels soon after colonization, and remain so throughout the lives of healthy, well-nourished hosts. The nonautochthonous members of the indigenous flora colonize the intestinal tract of animals living in a given area but may not be in all individuals of a given species (24). In mice and rats, the lactobacilli are among the autochthonous bacteria colonizing the nonsecreting gastric epithelia. Thus, certain bacteria appear to be specific for a given host species throughout life and are independent of environmental factors. Attachment of microorganisms to the intestinal wall seems to be mediated by acid mucopolysaccharides occurring on the surface of the bacteria (24). Microorganisms living in such intimate relationship with the host must produce physiological effects that involve the total ecological condition present, viz., the environment within the intestinal tract at different locations as well as interactions of the different members of the microflora. These may be subtle or obvious depending upon the individual conditions within any one given host. Autochthonous lactobacilli and bacteroides when inoculated into germ-free mice depressed the intestinal alkaline phosphatase (24). Physiological interactions with its autochthonous biota must have important consequences for the health and well-being of an animal. Photomicrographs of frozen sections of the intestinal wall show that microorganisms are associated closely with the epithelia of the gut wall and appear embedded in mucus adherent to the mucosa. These microorganisms are difficult to dislodge but evidently their overgrowth is prevented by removal during peristalsis (4).

Other than changes in the flora that result from changes in eating habits as a person ages, relatively little is known about the impact of moderate variations in the diet. Highly refined, digestible, and absorbable diets have small but significant effects on the bacterial flora of feces (26). A marked stability seems to be the dominant feature of the normal bacterial flora of the lower intestinal tract. The numbers of bifidobacteria are stable when other microorganisms increase and decrease in response to protein, fat, and carbohydrate in the diet. People on carbohydrate diets had significantly fewer bacteroides, more enterococci, and more aerobes than persons eating western diets that contained more meats (5). In studies on Ugan[dans], Japanese, and Indians, the fecal materia[l] contained lower concentrations of bile sal[ts] than that from western countries. On a chemi[cally] defined diet, the fecal mass was reduce[d] greatly since the diet was assimilated most[ly] (1). The total anaerobes remained the same a[s] before the intake of the synthetic diet but the bifidobacteria and lactobacilli were reduce[d] markedly.

The intestinal microflora is affected b[y] its interrelationships with bile acids in th[e] intestinal tract (7). Unconjugated (free) bil[e] acids are much more inhibitory than the con[jugated] forms. When intestinal bacteria decon[jugate] the bile salts, susceptible bacteria ar[e] then inhibited by the free acids. In a study o[f] two strains each of *C. perfringens*, *B. fragilis*, *Lactobacillus* sp., and enterococci, dihydrox[y] acids were more inhibitory than trihydrox[y] acids. The former were inhibitory at concentra[tions] of 1 to 2 mM and the trihydroxy at [...] mM. Larger numbers of microorganisms requi[r]ed higher concentrations for inhibition. The au[thors] suggested that deconjugation of bile salt[s] was a means for the autoregulation of th[e] bacterial population of the intestinal trac[t]. Metabolic activities of the intestinal bacter[ia] also have been associated with the occurren[ce] of cancer of the colon (6). It was hypothesize[d] that the cancer is caused by the production o[f] carcinogens and/or co-carcinogens from dieta[ry] components, or from intestinal secretions pro[duced] in response to the diet. Furthermore, th[e] nature of the diet affects composition of th[e] intestinal flora and substrates that are availabl[e] for bacterial action. In testing the hypothesis[,] bile acids (acid steroids) and neutral steroid[s] were studied. Many steroids have been carcino[genic] to animals. These steroids include deox[y]cholic acid, bis-norΔ^5 cholenic acid, apocholi[c] acid, and estradiol. The amount of bile acids i[n] feces is dependent on the amount of fat in th[e] diet. Initial studies compared the occurrence o[f] cancer in Scotland, England, and the USA wit[h] that in India, Japan, and Uganda. In the latte[r] three countries where the incidence of cance[r] was low, the number of bacteroides was lowe[r] and enterococci and lactobacilli were higher i[n] fecal specimens. Fecal steroids (neutral an[d] acid) were lower in specimens from Ugand[a,] India, and Japan. Also, the percent of steroid[s]

Toxic shock syndrome: An ecologic imbalance within the genital microflora of women?

CHRISTINE C. SANDERS, PH.D.
W. EUGENE SANDERS, JR., M.D.
JOAN E. FAGNANT, M.S.
Omaha, Nebraska

Epidemiologic data suggested that toxic shock syndrome (TSS) may be caused by an imbalance among the flora of the female genital tract. Since natural defense mechanisms often involve antagonistic interactions between the flora and potential pathogens, the ability of genital lactobacilli to inhibit *Staphylococcus aureus* was determined in agar overlay assays. Lactobacilli were chosen for study because previous investigations had suggested an important role for this genus in maintenance of health of the female genital tract. Fourteen of 50 strains of lactobacilli and Lactinex inhibited the growth of certain staphylococci, including strains from cases of TSS. The inhibitory activity of some lactobacilli was variable and could be enhanced by exogenously supplied substrates. Growth of one consistently inhibitory lactobacillus was inhibited by *Staphylococcus aureus*. A model for the etiology of toxic shock syndrome in menstruating women is proposed. The model includes antagonistic interactions between lactobacilli and staphylococci and the influence of tampons on these interactions to favor the staphylococcus. (AM. J. OBSTET. GYNECOL. 142:977, 1982.)

OVER THE LAST several years, the toxic shock syndrome has been recognized increasingly among women.[4, 11, 14] This syndrome, originally described by Todd and associates,[13] occurs primarily during menses. It is associated with the use of tampons and the presence of *Staphylococcus aureus* in the genital tract.[4, 11, 14] The precise sequence of events leading to this syndrome remains unknown. However, the observation that toxic shock syndrome, like trichomoniasis, candidiasis, and symptomatic gonorrhea, occurs most frequently during menses suggests that cycle-dependent changes in the genital flora may also play a role in susceptibility to this disease.

Since the late 1800s lactobacilli have been thought to play an active role in maintenance of health of the female genital tract. However, very little data concerning their protective role have been gathered over the ensuing nine decades. Recent studies in our laboratory have shown that endocervical lactobacilli may play a role in resistance of women to gonorrhea.[9] Certain strains of lactobacilli were found to be inhibitory to the growth of *Neisseria gonorrhoeae* in vitro. Their presence in vivo was associated with resistance to infection following exposure to infected consorts. This protection appeared to be maximal during the 2 weeks following menses, when the lactobacilli were most prevalent among the flora. It was minimal during menses when the lactobacilli were least prevalent.

During the same study, a similar inverse relationship between prevalence of lactobacilli and staphylococci was also observed.[9] Like *Neisseria gonorrhoeae*, all staphylococci were most prevalent in the endocervix of women cultured the week preceding and the week of menses. Although not specifically shown, endocervical carriage rates for *Staphylococcus aureus* followed this same pattern. These epidemiologic data suggested that the endocervical lactobacilli may play a role in resistance to toxic shock syndrome. Thus, the current study was designed to investigate this possibility further. Since an

From the Department of Medical Microbiology, Creighton University School of Medicine.

This research was supported by Grant No. AI-11584 from the National Institute of Allergy and Infectious Diseases.

Received for publication June 3, 1981.

Revised September 28, 1981.

Accepted December 2, 1981.

Reprint requests: Dr. Christine C. Sanders, Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178.

0002-9378/82/080977+06$00.60/0

Table III. Effect of the nonbacterial components in Lactinex on the inhibitory activity of its component lactobacilli against *Staphylococcus aureus,* TSS 1

	Inhibitory activity of component mixed with			
Nonbacterial component	*Water*	L. acidophilus-L	L. bulgaricus-L	*Both strains*
None	−*	−	−	−
Milk	−	+†	−	+
Whey	−	−	−	+
Sugar	−	−	−	−
Milk plus whey	−	+	−	+
Milk plus sugar	−	+	−	+
Whey plus sugar	−	−	−	+
Milk plus whey plus sugar	−	+	−	+‡

*Preparation consistently noninhibitory.
†Preparation consistently inhibitory.
‡Contains all the components of Lactinex granules.

Table IV. Influence of evaporated milk on the inhibitory activity of lactobacilli against *Staphylococcus aureus* recovered from cases of toxic shock syndrome

	Target Staphylococcus aureus		
Milk plus effector	*TSS 1*	*TSS 2*	*TSS 3*
L. casei var *rhamnosus* 2	−*	+†	−
L. cellobiosus 8	+	−	−
L. acidophilus 15	+	+	+
L. acidophilus 16	−	+	+
L. acidophilus 17	+	+	+
L. leichmannii 22	−	−	+
L. leichmannii 25	−	−	+
L. jensenii 41	+	+	+
L. acidophilus-L	+	+	+

*Effector consistently noninhibitory with and without milk.
†Effector consistently noninhibitory without milk and consistently inhibitory with milk.

For these tests, the three *S. aureus* TSS strains were used as targets. Among the endocervical isolates milk enhanced the activity of eight strains that had shown no inhibition of the TSS isolates in initial tests (Table IV). This enhanced activity was not always uniform, as not all of the TSS targets were inhibited by each strain when tested with milk. Milk had no effect on the ATCC strains, nor did it alter the activity of isolates that were active against the TSS targets initially (Table II). Effectors that were inhibitory remained inhibitory, and effectors that were variable in their activity remained variable.

In a final series of tests, the inhibitory activity of *Staphylococcus aureus* against lactobacilli was determined by means of the reverse agar overlay assay. For these tests all 12 *S. aureus* strains were used as effectors and nine isolates of lactobacilli were used as the targets. The lactobacilli included two strains consistently noninhibitory for *S. aureus,* two strains consistently inhibitory for *S. aureus,* three strains of variable activity against *S. aureus,* and two strains inhibitory for *S. aureus* only when tested in the presence of milk. Only one target lactobacillus, *L. jensenii* 43, was inhibited by the 12 *S. aureus* effectors. However, this was one of the two strains that had been consistently inhibitory for *S. aureus* (Table II). None of the other lactobacillus targets was inhibited by any of the 12 *S. aureus* effectors.

Comment

The results of this study indicated that lactobacilli are capable of inhibiting the in vitro growth of *Staphylococcus aureus.* Although only 14 of the 50 (28%) strains exhibited some inhibition of the *S. aureus* targets, the active strains represented those species most commonly found in the genital flora of women.[2, 8] These included *L. acidophilus, L. leichmannii,* and *L. jensenii*. The antagonistic activity was consistent for some strains of lactobacilli, inconsistent for others and enhanced by exogenously supplied substrates (e.g., milk) in still other strains. Although delineation of the mechanism responsible for the inhibition observed was beyond the purpose of this paper, it is unlikely that the production of an acidic pH was the sole mechanism involved. Since lactobacilli are highly acidogenic, most strains tested should have been consistently inhibitory for the acid-intolerant *N. gonorrhoeae*. This was not the case. Also, many lactobacilli, including *L. acidophilus,* do not produce acid in the presence of milk. Thus, milk should not have enhanced the inhibitory activity against *Staphylococcus aureus,* were the mechanism acid mediated. Finally, the use of a highly buffered overlay medium in the assay was designed to minimize the effects of acid produced in the lower agar layer by effector strains.

The results obtained in this study clearly underscore the overall variable nature of the antagonistic potential of lactobacilli for *Staphylococcus aureus*. This variability in and of itself may ultimately help to explain the etiology of toxic shock syndrome in menstruating women. For toxic shock syndrome to occur, the environment of the genital tract must be conductive for the establishment, proliferation, and/or toxin production by *Staphylococcus aureus*. From epidemiologic studies, it appears that the environment in most women is not conductive for either the establishment or the proliferation of *Staphylococcus aureus*.[4, 5, 7] These data suggest the presence of some natural defense mechanism operative in most women. This defense mechanism appears to be greatest in nonmenstruating women and lowest but not absent in women genitally colonized with *Staphylococcus aureus*, especially during menses. Thus, for toxic shock syndrome to occur, the defense mechanism operative in colonized women must be nullified.

Results of this and previous studies suggest that the natural defense mechanism may involve the genital lactobacilli. In some women the use of tampons, especially continuous use of superabsorbent tampons, may remove substrates from the environment that are essential for the production of the inhibitory effect. Such substrates might normally be provided by cervical mucus or the menstruum itself. Conversely, in women harboring lactobacilli consistently inhibitory for *Staphylococcus aureus*, use of tampons, regardless of type or duration of use, would be unlikely to nullify the protective effect. This model might also explain the recurrence of toxic shock syndrome in women.[4] Such women may be chronic carriers of staphylococci whose proliferation and/or toxin production are prevented by the lactobacilli during most of the menstrual cycle. However, with each onset of menses and use of tampons, the protective effect is nullified and the disease recurs. Thus, in this model, the ability of the staphylococci to proliferate and/or produce toxin and initiate disease depends upon the precise interaction of the lactobacilli, tampons, and the staphylococci and their ultimate effect on the environment of the genital tract.

Clearly more studies are required to test this model directly. Conditions for optimizing the antagonistic effect of the lactobacilli need to be defined, and the influence of the lactobacilli on toxin production by staphylococci needs to be examined. The antagonistic potential of other organisms among the genital flora must also be studied. However, previous studies on the protective activities of the genital flora against a variety of diseases have singled out the lactobacilli as the most important component of the flora.[9, 12, 15] In fact, several investigators have reported the successful use of lactobacillus preparations in treatment of various genital infections.[3, 6, 10, 12, 16]

If the validity of this model is supported by results of future studies, then direct measures may be taken to prevent toxic shock syndrome. These may include artificial replacement or enhancement of the protective lactobacilli during menses and might be accomplished through the use of lactobacillus-containing douches or lactobacillus-seeded tampons during menses. The demonstration in this study of the inhibitory activity of Lactinex against *Staphylococcus aureus* suggests that such commercial preparations may ultimately prove useful in this approach to prevention.

We thank Laurier P. Couture for technical assistance.

REFERENCES

1. Buchanan, R. E., and Gibbons, N. E.: Bergey's Manual of Determinative Bacteriology, ed. 8. Baltimore, 1974, The Williams & Wilkins Company.
2. Carlsson, J., and Gothefors, L.: Transmission of *Lactobacillus jensenii* and *Lactobacillus acidophilus* from mother to child at time of delivery, J. Clin. Microbiol. **1:**124, 1975.
3. Collins, E. B., and Hardt, P.: Inhibition of *Candida albicans* by *Lactobacillus acidophilus*, J. Dairy Sci. **63:**830, 1980.
4. Davis, J. P., Chesney, P. J., Wand, P. J., and LaVenture, M.: Toxic shock syndrome. Epidemiologic features, recurrence, risk factors, and prevention, N. Engl. J. Med. **303:**1429, 1980.
5. Galask, R. P., Larsen, B., and Ohm, M. J.: Vaginal flora and its role in disease entities, Clin. Obstet. Gynecol. **19:**61, 1976.
6. Gunston, K. D., and Fairbrother, P. F.: Treatment of vaginal discharge with yoghurt, S. Afr. Med. J. **49:**675, 1975.
7. Osborne, N. G., Wright, R. C., and Grubin, L.: Genital bacteriology: a comparative study of premenopausal women with postmenopausal women, Am. J. Obstet. Gynecol. **135:**195, 1979.
8. Rogosa, M., and Sharpe, M. E.: Species differentiation of human vaginal lactobacilli, J. Gen. Microbiol. **28:**197, 1960.
9. Saigh, J. H., Sanders, C. C., and Sanders, W. E., Jr.: Inhibition of *Neisseria gonorrhoeae* by aerobic and facultatively anaerobic components of the endocervical flora: Evidence for a protective effect against infection, Infect. Immun. **19:**704, 1978.
10. Sandler, B.: Lactobacillus for vulvovaginitis, Lancet **2:**791, 1979.
11. Shands, K. N., Schmid, G. P., Dan, B. B., Blum, D., Guidotti, R. J., Hargrett, N. T., Anderson, R. L., Hill, D. L., Broome, C. V., Band, J. D., and Fraser, D. W.: Toxic-shock syndrome in menstruating women. Association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases, N. Engl. J. Med. **303:**1436, 1980.

these 40 chronic carriers, who were mainly adults, were HBeAg-positive and their HBsAg titres were generally lower than those found in more recently infected children. On this evidence many adult carriers may be less infective than *e* antigen child carriers but they may experience the consequences of chronic virus infection, such as cirrhosis and hepatoma, in later life. All 4 patients with primary liver cancer carried HBsAg in their blood at diagnosis and 2 were known to have carried the virus for at least 7 years.

This survey gives a guide as to the best age at which to use hepatitis B vaccine in the Gambia. Infants should receive the vaccine around the age of 2 months when chances of infection are low perhaps because maternal antibody is present. Such young children are known to have a normal response to the vaccine[11] so this new and highly effective vaccine could be given along with the diphtheria/pertussis/tetanus and oral polio vaccines which are now widely and successfully given during the first 6 months of life in The Gambia. However, there is no proof that hepatitis B vaccine will lower the incidence of cirrhosis and hepatoma. This effect must be established before the widespread use of an expensive vaccine in a poor country is considered. HBV causes only subclinical infection in most of those affected.

We thank the Chief of Manduar and Alkali Minte in Keneba for help. Mr K. Williams, Mr K. Jamneh, and Mr S. Rahman gave expert technical assistance. We also thank Dr R. Whitehead, Dr A. Prentice and Dr B. M. Greenwood for their enthusiastic help and advice.

Correspondence should be addressed to H. C. W.

REFERENCES

1. Ryder RW. A proposal for a long term controlled trial of hepatitis B virus vaccine in The Gambia to prevent hepatitis and liver cancer. Paper submitted to the Gambian Medical and Health Department 1982.
2. Barin F, Perrin J, Chotard J, Denis F, N'Doye R, Diopmar I, Chiron J-P, Coursaget P, Goudeau A, Maupas P. Cross-sectional and longitudinal epidemiology of hepatitis B in Senegal. *Prog Med Virol* 1981; **27:** 148–62.
3. Prince AM, White T, Pollock N, Riddle J, Brotman B, Richardson L. Epidemiology of hepatitis B infection in Liberian infants. *Infect Immun* 1981; **32:** 675–80.
4. Deinhardt F. Predictive value of markers of hepatitis virus infection. *J Infect Dis* 1980; **141:** 299–305.
5. Billewicz WZ, McGregor IA. The demography of two West African (Gambian) villages, 1951–75. *J Biosoc Sci* 1981; **13:** 219–40.
6. McGregor IA. Health and communicable disease in a rural African environment. *Oikos* 1976; **27:** 180–92.
7. Stevens CE, Beasley RP, Tsui J, Lee W-C. Vertical transmission of hepatitis B antigen in Taiwan. *N Engl J Med* 1975; **292:** 771–74.
8. Beasley RP, Trepo C, Stevens CE, Szmuness W. The 'e' antigen and vertical transmission of hepatitis B surface antigen. *Am J Epidemiol* 1977; **105:** 94–98.
9. Prince AM, Metselaar D, Kafuko GW, Mukwaya LG, Ling CM, Overby LR. Hepatitis B antigen in wild-caught mosquitoes in Africa. *Lancet* 1972; ii: 247–53.
10. Wills W, Larouze B, London WT, Millman I, Werner BG, Ogston W, Poutaghia M, Diallo S, Blumberg BS. Hepatitis B virus in bedbugs (*Cimex hemipterus*) from Senegal. *Lancet* 1977; ii: 217–19.
11. Barin F, Gourdeau A, Denis F, Yvonnet B, Chiron J-P, Coursaget P, Diomar I. Immune response in neonates to hepatitis B vaccine. *Lancet* 1982; i: 251–53.

Nutrition: The Changing Scene

FERMENTATION IN THE HUMAN LARGE INTESTINE: EVIDENCE AND IMPLICATIONS FOR HEALTH

JOHN H. CUMMINGS

Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Trumpington Street, Cambridge CB2 1QE

THE principal functions of the large intestine are to conserve water and electrolytes secreted into the gut during digestion, to provide a controllable route for the excretion of waste products of metabolism and of toxic substances, and to contain safely the microorganisms that, by fermentation, conclude the digestive process. Fermentation, the anaerobic breakdown of carbohydrate, is an important but hardly recognised part of human digestive physiology. It has a direct bearing on salt and water absorption from the colon, on bowel habit, on the excretion of toxic substances, and on nitrogen and sterol metabolism, and it may also influence intermediary metabolism in the colonic epithelium, liver, and peripheral tissues.

EVIDENCE OF FERMENTATION

In animal physiology the word fermentation is conventionally used to describe the reaction in which carbohydrate, usually polysaccharide, is broken down by the concerted action of many species of anaerobic microorganisms (see accompanying figure). The end-products of this process are short-chain (or volatile) fatty acids, principally acetic, propionic, and butyric acids, and the gases carbon dioxide, methane, and hydrogen. Through fermentation the microorganisms obtain energy for growth. What is the evidence that this process occurs in the human colon?

Anaerobic Microflora

The large intestine of human beings contains a luxuriant mixed culture of bacteria, 99% of which are anaerobes.[1] Most of these organisms are saccharolytic—ie, they derive their energy primarily from carbohydrate and its derivatives.[2] The number of organisms in colonic and faecal material has been estimated at 10^{10}–10^{11}/g, which means that we each have more microbial than human cells. Difficulties in estimating microbial size, however, and the extremely diverse microbial ecology in the colon mean there is little information on the actual mass of microbial matter present. Early estimates put the microbial mass at 25% of colonic solids, but more recent methods indicate that it may be much higher, between 40% and 55%[3,4] (ie, about 200 g microbial matter[5]). The implications of the presence of such a large anaerobic organ in man have never been fully explored.

Available Substrates

The main carbohydrate passing from the ileum to the caecum is the polysaccharide fraction of plant cell walls (dietary fibre) together with some starch that may also escape digestion. Only very small quantities of simple sugars are not absorbed from the small intestine in healthy people, unless they are lactase deficient. The main source of endogenous carbohydrate secreted into the colon is mucus. Intestinal glycoproteins are 80% carbohydrate,[6] and the human colon contains microorganisms capable of degrading these molecules.[7]

It is difficult to define what amounts of these substrates are available to the microflora. Average intake of the fermentable component of dietary fibre is 15–20 g/day in the UK. When this diet is eaten under controlled conditions 3–5 g of this component appears undegraded in the faeces.[8] The amount of starch that escapes breakdown in the small bowel is currently being investigated. Using the evolution of hydrogen in breath as a marker of colonic fermentation, Levitt and colleagues[9,10] estimate that 10–15% of the starch in foods such as oats, white bread, and potatoes escapes methane and carbon dioxide, with hydrogen appearing only under abnormal conditions (such as low pH due to rapid breakdown of soluble carbohydrate) which also favour the production of ethanol. However, only a minority of human beings (30–50%) in Europe and North America produce methane and almost all eliminate hydrogen during fermentation.[25,26] No great physiological importance seems to be attached to the production of these gases, although they may give rise to abdominal distension and pain which often lead patients to seek medical advice. The answer to the question of why hydrogen rather than methane should be produced in so many people may yield new insights into colon metabolism.

Microflora

Bacteria in the colon metabolise carbohydrate in order to obtain energy for growth and maintenance. Maintenance energy is a complex concept but it includes energy for motility, enzyme synthesis, maintenance of ionic and osmotic gradients, and active transport. Maintenance energy is a much smaller fraction of total energy used in microorganisms than in mammalian tissue of similar metabolic size, but can account for about a third of available energy. One of the effects of fermentation is to stimulate microbial growth in the colon. When human beings are fed fermentable carbohydrate faecal microbial mass increases.[27] The yield of microbial cells from fermented carbohydrate has been studied in some detail in ruminants.[28] The yield of cells (dry weight) is around 20–45% of the weight of carbohydrate fermented. When similar calculations are applied to human studies cell yields of 20–30% are found.[29] This finding is one of several indications of the parallel which exists between rumen and large-intestinal fermentation. The stimulation of microbial growth is partly responsible for the increase in stool mass due to fermentable carbohydrate such as dietary fibre.[27]

To grow, bacteria require nitrogen. Potential sources of nitrogen in the colon include urea, which is hydrolysed by microbial urease to ammonia, and dietary or endogenous protein. The relative contributions of these sources to microbial protein synthesis in human beings are unknown, but the net effect of feeding fermentable carbohydrate to healthy subjects is to increase faecal nitrogen output.[27] A significant fraction of this increase is accounted for by greater excretion of microbial cells.[30] Fermentation in the colon has the effect, therefore, of diverting nitrogen into microbial protein synthesis. Faecal ammonia levels fall in human volunteers when they are fed fermentable carbohydrate.[31] In patients with hepatic cirrhosis lactulose (which is fermented in the colon) given by mouth leads to a 2–3-fold increase in faecal nitrogen excretion and a decrease in urea production and urea pool size.[32] The fall in urea production was believed to be due to reduced portal-blood ammonia. Ammonia is also an important regulator of cell metabolism and may be involved in carcinogenesis.[33]

Steroid Metabolism

Fermentation may affect many other reactions in the colon ascribed to the bacteria. Steroid 7-α-dehydroxylase activity is inhibited by low pH[34] and when fermentation is stimulated in man by giving lactulose the pH falls and the proportion of deoxycholic acid in bile falls significantly.[35] Studies in germ-free rats[36] have shown that the intestinal microflora is an important metabolic organ of the body affecting the immune system, resistance to infection, and steroid, mucin, and gut enzyme metabolism. The microorganisms possess several steroid-hormone-metabolising enzymes which can affect th proportions of steroid metabolites in the gut, leading changes in urinary levels.[37] The detoxification of xenobiotic such as propachlor (a potential carcinogen) is also influenced by the microflora[38] because the necessary steps prolong thei life in the enterohepatic circulation where the reactions can b affected by fermentation patterns.

Control of Fermentation

Unlike other organs of the body the microflora is not under direct neural or hormonal control. Control must be effected through the supply of both endogenous and exogenous substrate, therefore, the amount and type of such material available need to be identified. Mucus, the main endogenous carbohydrate source for bacteria is known to influence colonic microbial metabolism.[39] There are pronounced differences in the variety of species carried by individuals. The mixture of species is likely to alter significantly the end-products of fermentation.[40] The turnover or transit time of material through the gut may also be important: in ruminants the turnover time of rumen contents significantly influences the efficiency with which microorganisms grow on a given carbohydrate.[28] The shorter the turnover time (within reason) the more efficient is growth because microbial pool size in the gut is reduced and less energy is required for maintenance.

REFERENCES

1. Moore WEC, Cato EP, Holdeman LV. Some current concepts in intestinal bacteriology. *Am J Clin Nutr* 1978; **31:** S33–42.
2. Miller TL, Wolin MJ. Fermentations by saccharolytic intestinal bacteria. *Am J Clin Nutr* 1979; **32:** 164–72.
3. Moore WEC, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol* 1974; **27:** 961–79.
4. Stephen AM, Cummings JH. The microbial contribution to human faecal mass. *J Med Microbiol* 1980; **13:** 45–56.
5. Banwell JG, Branch W, Cummings JH. The microbial mass in the human large intestine. *Gastroenterology* 1981; **80:** 1104.
6. Allen A. Structure and function of gastrointestinal mucus. In: Johnson LR, ed. Physiology of the gastrointestinal tract. New York: Raven Press, 1981: 617–39.
7. Salyers AA, Vercellotti JR, West SEH, Wilkins TD. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. *Appl Environ Microbiol* 1977; **33:** 319–22.
8. Cummings JH. Dietary fibre. *Br Med Bull* 1981; **37:** 65–70.
9. Anderson IH, Levine AS, Levitt MD. Incomplete absorption of the carbohydrate in all-purpose wheat flour. *N Engl J Med* 1981; **304:** 891–92.
10. Levine AS, Levitt MD. Malabsorption of starch moiety of oats, corn and potatoes. *Gastroenterology* 1981; **82:** 1209.
11. Stephen AM, Haddad AC, Phillips SF. Dietary and endogenous sources of carbohydrate substrate in the human colon. *Gut* 1981; **22:** A893.
12. Wolf MJ, Khoo U, Inglett GE. Partial digestibility of cooked amylomaize starch in humans and mice. *Die Starke* 1977; **29:** 401–05.
13. Vercellotti JR, Salyers AA, Wilkins TD. Complex carbohydrate breakdown in the human colon. *Am J Clin Nutr* 1978; **31:** S86–89.
14. Cummings JH. Short chain fatty acids in the human colon. *Gut* 1981; **22:** 763–79.
15. Rubinstein R, Howard AV, Wrong OM. In vivo dialysis of faeces as a method of stool analysis. IV. The organic anion component. *Clin Sci* 1969; **37:** 549–64.
16. McNeil NI, Cummings JH, James WPT. Short chain fatty acid absorption by the human large intestine. *Gut* 1978; **19:** 819–22.
17. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG. Absorption of short chain fatty acids by the colon. *Gastroenterology* 1980; **78:** 1500–07.
18. Roediger WEW. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 1980; **21:** 793–98.
19. Bergman EN. Production and utilization of metabolites by the alimentary tract as measured in portal and hepatic blood. In: McDonald IW, Warner ACI, eds. Digestion and metabolism in the ruminant. Australia: University of New England Publishing Unit, 1975: 292–305.
20. Anderson JW, Bridges SR. Short chain fatty acid metabolites of plant fiber alter glucose metabolism of isolated rat hepatocytes. *Am J Clin Nutr* 1982; **35:** 840.
21. Williamson JR, Martin-Requero A, Corkey BE, Brandt M, Rothman R. Interactions between α-ketoisovalerate, propionate and fatty acids on gluconeogenesis and ureogenesis in isolated hepatocytes. In: Walser M, Williamson JR, eds. Metabolism and clinical implications of branched chain amino and ketoacids. Amsterdam: Elsevier North Holland, 1981: 105–17.
22. Brockman RP. Insulin and glucagon responses in plasma to intraportal infusions of propionate and butyrate in sheep (*Ovis bries*). *Comp Biochem Physiol* 1982; **73A:** 237–38.
23. Kruh J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol Cell Biochem* 1982; **42:** 65–82.

References continued on next page

ocardium; of
of the tissue,

it might be
part of the
of occurrence

his case as a
of which is
n the domain

BACILLUS ACIDOPHILUS AND ITS THERAPEUTIC APPLICATION

LEO F. RETTGER, Ph.D., and HARRY A. CHEPLIN, Ph.D.
NEW HAVEN, CONN.

Bacillus acidophilus was first observed and described in 1900 by Moro.[1] It is a rather large gram-positive bacterium which is quite pleomorphic and which in many respects resembles Massol's *Bacillus bulgaricus* and Tissier's *Bacillus bifidus.* It was claimed by Moro to be the chief inhabitant of the intestine of infants that subsist entirely on mother's milk. This assertion was disputed by Tissier[2] who protested that *B. bifidus* holds the place of prime importance. *B. acidophilus* and *B. bifidus* are now known to constitute the main flora of the breast-fed child, the latter being, perhaps, the more prominent of the two. As the diet changes and becomes more and more complex, there is a corresponding change in the kinds and relative numbers of intestinal bacteria, until finally the intestinal population assumes the character of that of the ordinary adult. *B. acidophilus and B. bifidus* gradually disappear to such an extent that their presence can be demonstrated in the feces with considerable difficulty only. Their place has been taken by various other organisms, some of which are decidedly fermentative and putrefactive, and, according to Metchnikoff and others, assume a rôle harmful to the host.

B. acidophilus is practically indistinguishable from *B. bulgaricus* and has undoubtedly often been mistaken for the latter. There are, however, two well-known points of distinction between these two organisms. *B. acidophilus* produces relatively little acid in milk (less than 1 per cent.) even after continued incubation, whereas *B. bulgaricus* may produce as much as 3 per cent. Furthermore, *B. acidophilus* attacks maltose with acid formation, while *B. bulgaricus* has no action on this sugar. The most important mark of difference is that relating to intestinal implantation. *B. bulgaricus,* as numerous experiments have shown, is unable to live and multiply in the intestine of the white rat and of man, whereas *B. acidophilus* undergoes rapid development when administered by mouth, or as the result of milk, lactose or dextrin feeding.

Rettger and Horton[3] and Hull and Rettger[4] had shown that the feeding of milk or lactose to experiment animals, including the white

1. Moro, E.: Ueber den *Bacillus acidophilus,* Jahrb. f. Kinderh. **52**:38, 1900.
2. Tissier, H.: Réchérches sur la flore intestinale normale et pathologique du nourisson, Thése, Paris, 1900.
3. Rettger, L. F., and Horton, G. D.: A Comparative Study of the Intestinal Flora of White Rats Kept on Experimental and on Ordinary Mixed Diets, Centralbl. f. Bakteriol. **73**:362, 1914.
4. Hull, T. H., and Rettger, L. F.: The Influence of Milk and Carbohydrate Feeding on the Character of the Intestinal Flora. J. Bacteriol. **2**:47, 1917.

not take other food in any form retained the acidophilus milk and complained of no distressing or otherwise injurous effect.

Chronic Constipation.—The two following cases of chronic constipation are briefly reviewed here, as they happened to be the first to take the treatment, and were among the most obstinate advanced cases of alimentary toxemia that have as yet come under our observation.

Case 1.—Subject D. had suffered from constipation for at least seven or eight years, and complained of fulness of the abdomen and of gas and pain in the epigastric region, also of an almost constant headache, visual disturbance, general discomfort after meals, loss of energy and initiative, general malaise, and what was most disturbing to him, melancholia and other indications of lessened mentality. He regularly required unusually large doses of laxative to induce bowel evacuation, and stated that he had been absolutely dependent on cathartics for at least two years for relief which was even then only partial and of very short duration.

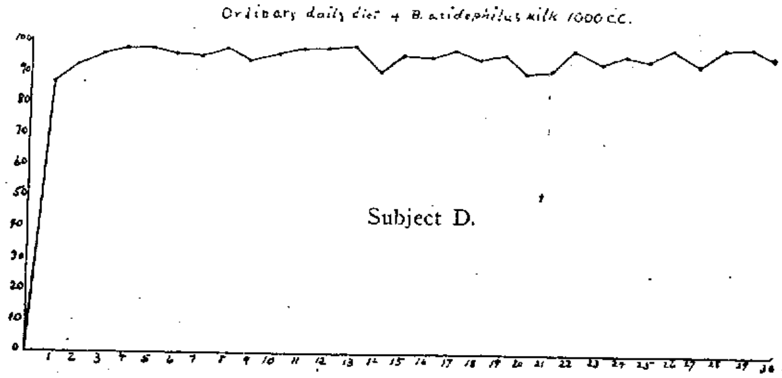

Fig. 1.—Results of addition of *B. acidophilus* milk to diet.

The subject was given 1 liter acidophilus milk daily, and instructed, as were all others, not to employ a cathartic, and to bring daily specimens (when possible) of stool. He reported almost daily at the laboratory for observation. Unfortunately, no sample of stool could be obtained before the beginning of the treatment. However, in none of the numerous experiments already conducted on man was *B. acidophilus* observed in the feces before treatment with *B. acidophilus* culture or special carbohydrates without much difficulty and then only rarely and in very small numbers; hence, it is safe to assume that in cases of constipation at least the per cent. of cultivable *B. acidophilus* cells is, without special stimulation, at or near 0.

Within forty-eight hours after the first ingestion of the milk culture the bacteriologic examination of the feces revealed a marked transformation of the flora with a preponderance of *B. acidophilus*-like organisms. By the end of the fourth day the gas producing organisms had apparently completely disappeared from the intestine, and the aciduric type reached a percentage level of at least 90 which it maintained throughout the course of the treatment (Fig. 1). The administration of the acidophilus milk had to be discontinued and the experiment ended after a period of thirty days, owing to the seasonal closing of the laboratory. The subject left very soon after for continued residence in the Orient.

ment resulted in temporary cure. Normal formed stools for the first time since 1909. Recurrence after four weeks; acute abdominal pain and diarrhea. Feces examination negative. Condition much improved after four months' residence in Norway. Partial recurrence in England (December, 1920, to April, 1921). Took ship for America April 2, 1921. Apparently normal on ship and for a few days after arrival in New Haven.

Within a week after subject B. reached New Haven he was suddenly seized with an attack of profuse diarrhea and acute pains in the descending colon. Presented himself for treatment in April. Stools were watery, very dark, and of pronounced putrefactive odor. By the third day the stools appeared lighter (yellowish) in color and less watery. After one week's treatment the abdominal pains had practically disappeared, the feces rapidly approached the normal and the diarrheal condition had almost completely ceased. During the second week's treatment he pronounced himself fully recovered. He continued apparently normal for about three weeks when owing to dietary indiscretion there was a slight return of the disturbance. The night before the partial recurrence he attended a banquet and indulged freely in all that was set before him, including two courses of meat. This setback lasted only two or three days.

Subject B took one quart of acidophilus milk daily from the first without any intermission. Except for the above mentioned and a second slight recurrence early in June brought on apparently by bathing in cold sea water, he was, according to all appearances and his own claims, in normal condition for two months and to the time when the experiment was interrupted owing to the closing of the laboratory (July 1). The subject has on several occasions since then informed the writers that he has suffered no recurrence.

Another subject whose case in some respects was similar to this reacted favorably to the treatment. A full history of this case, with results, will be given in a later paper.

Colitis.—The two cases of colitis which came under our observation were of the acute type. One was apparently uncomplicated, but had a long history of intestinal disturbance. The other was complicated with nephritis. The former responded completely to the milk treatment (800 c.c. daily) and after about one month returned to his work. He continued in apparently good health for about two months when he experienced a partial reversion, due to overconfidence in his improved condition and indulgence in late shore dinners.

The second patient gave every indication of physical improvement when, owing to his serious nephritic condition, he required special hospital treatment and was compelled to discontinue the use of the acidophilus milk. His severe abdominal pains had almost completely disappeared, however, and he was suffering apparently very little from the colitis.

Both of these cases will be presented more fully at a later date.

Sprue.—Case 4.—Subject Wr had contracted sprue while in China. Though the affection was not of an acute type, it was serious and compelled him to return to the United States where for at least two years he was constantly under its annoying and debilitating influence. He took one quart of the acidophilus milk daily for six weeks during which time the character of the feces changed completely from the clay-colored, soft and extremely offensive type to the yellow, almost formed and almost odorless. The gas disappeared from the colon, and the subject stated from day to day that he thought he was for the time at least in perfectly normal condition. The bacteriological results of fecal examination are shown in Figure 2.

A second case of sprue was that of a returned missionary who had contracted the disease during twenty years of service in China. When seen by the senior author he had been confined to his bed for several months with the characteristic symptoms in their most acute form, including tetany. He was emaciated and subject to abdominal pains and gaseous distension.

This patient has been under observation for almost six months, during which period he has taken, with very few brief intermissions, one quart of acidophilus milk daily (the milk being sent to him by special messenger). He has shown from almost the beginning gradual improvement in his condition, though he has from time to time suffered relapses.

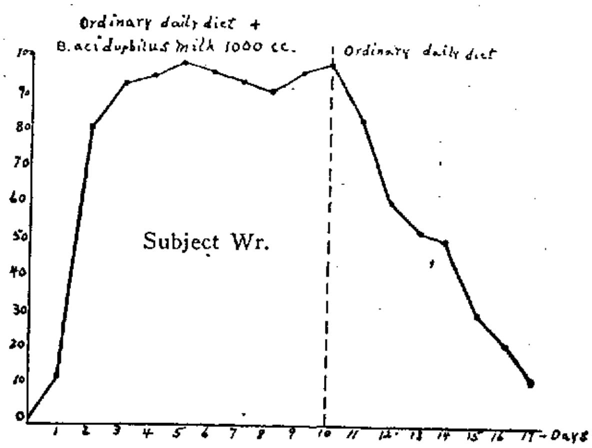

Fig. 2.—Results of addition of *B. acidophilus* milk to ordinary diet and then returning to ordinary diet.

Dermatitis.—All of the three cases of dermatitis were those of eczema. Treatment of two of them was discontinued before any definite results could be obtained. The third patient (Subject Q) responded completely to the treatment, and for five months has been free from the eczema which for at least twelve years had been a source of constant annoyance and embarrassment. When first seen practically the entire face was involved, as well as other parts of the body.

Transformation of the intestinal flora was effected with considerable difficulty, and required fully a month. However, by increasing the daily amount of added lactose from 50 to 100 gm. a high aciduric flora was established and maintained throughout the remainder of the experiment. Concomitant with this change there began a clearing of the skin which continued until after another month almost all traces