Diarrheal Episodes and Diarrheal Disease: 
Acute Disease with Chronic Implications

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ABSTRACT

Diarrheal episodes and diarrheal disease are often considered to be acute events of limited duration; a review of current literature indicates that this is not true. Diarrheal episodes caused by many bacteria, viruses, protozoans and other parasites cause alteration of intestinal structure and function. Consequences of such diarrhea-associated gut alterations include loss of normal defense mechanisms against secondary opportunistic pathogens and the ability to exclude macromolecules from systemic circulation. Additionally, loss of endogenous nutrients and malabsorption of essential nutrients result from diarrheal episodes; the consequences of such losses, even of a single essential nutrient, is compromised immune function, which predisposes to further infection. The net result of such events in some persons is long-term debilitating disease(s) such as allergy, autoimmune disorders and neoplasia.

The general perception of diarrheal episodes in highly developed industrialized nations is that they are acute, sporadic events. Even diarrheal disease in such nations is considered to be self-limiting and therefore of no consequence with regard to long-term, debilitating disease. This paper discusses foodborne diarrhea-causing agents, and through literature review, presents evidence that diarrhea may predispose an individual to diseases with long-term consequences: allergy, autoimmune disorders, potentially lethal infections by opportunistic pathogens, malnutrition and neoplasia.

INCIDENCE OF FOODBORNE DIARRHEAL DISEASE

The true incidence of foodborne diarrheal disease (or diarrheal disease of any etiology) is nearly impossible to determine. Of the large outbreaks of foodborne illness reported to the Centers for Disease Control (CDC) from 1972 to 1978, the etiology was confirmed in only 38% of the outbreaks (52). Some foodborne diseases are reportable to CDC by state health officials on a passive, voluntary basis; these are botulism, salmonellosis, shigellosis, campylobacteriosis, typhoid fever and any suspected food-associated illness (Mark Finch, CDC, personal communication, 1983). However, outbreaks may go unreported for a variety of reasons. First, since many bacterially mediated food poisonings or infections are of limited duration, people tend not to report them. Second, of those who do seek a physician's opinion because the diarrhea is severe or the episode prolonged, most are treated symptomatically and diagnostic tests are seldom done. Third, even when the episode(s) is (are) severe enough to require hospitalization, if a hospital is equipped to diagnose infections from newly recognized pathogens such as Yersinia enterocolitica or Aeromonas hydrophila, these tests may not always be done. Finally, suspect food is seldom available for analysis by the time the epidemiology indicates illness of foodborne origin. The food history questionnaire then assumes tremendous importance, as does the memory of the patient, and this too has been shown to be subject to error (34).

One reason for the underreporting of diarrhea is that the public considers diarrhea to be a self-limiting, unpleasant nuisance, but in most instances certainly not life-threatening and with few long-term health consequences. As may become apparent, this is not true.

PROTECTIVE MECHANISMS OF THE GASTROINTESTINAL (GI) TRACT

A review of the immunologic defense mechanisms of the gut would be too lengthy to include in this paper; the reader is directed to other reviews (4,54) of this subject. Basically, the gut defense mechanisms may be divided into local immunologic defenses and nonimmunological factors. Immunologic defenses include secretory IgA (slgA) (reviewed in ref. 55), cell-mediated immunity and other immunoglobulins (IgM, IgG, IgE). slgA is the principal immunoglobulin active along the mucosal linings of the gut and elsewhere. When slgA is lacking, IgM may serve the same functions, but not as efficiently. slgA is considered to be more resistant to enzymatic degradation. Owing to the special structure that joins the two IgA molecules, the secretory component, slgA remains associated with the glycolcalyx of intraluminal epithelial cells of the gut. This property ensures that antibody activity remains along the mucous covering of the epithelium where it is needed rather than floating free in the lumen where it may be degraded. The principal functions of slgA are to: (a) prevent colonization or inva-
by bacteria by preventing contact with the epithelium, (b) neutralize viruses and harmful microbial metabolites and (c) prevent absorption of macromolecules (food constituents, etc.), thus facilitating their digestion (54). SlgA does not fix complement, the consequences of which would be local tissue damage.

Nonimmunological defense factors include physical barriers such as mucus from the goblet cells and the epithelium. Additionally, nonphysical barriers or factors include competitive indigenous microflora, other GI secretions, peristaltic movement and interferons. The mucus from the goblet cells is a physical barrier in the sense that potentially pathogenic organisms must penetrate the mucus to reach the epithelium. Organisms that produce mucinase are far more effective colonizers than are nonproducers (25). Motility of an organism also seems to be important to the organism's ability to penetrate the mucous barrier and reach the epithelium; this has recently been demonstrated for Vibrio cholerae (61) and C. jejuni (36). Likewise, adhesins or other adhesive or attachment factors which mediate attachment to the mucosa facilitate colonization or invasion by numerous organisms. During periods of increased microbial growth in the gut or increased production of macromolecules by microorganisms, formation of immune complexes increases. Immune complexes of this type can trigger additional mucus production and release by goblet cells, thus thickening the barrier, a defense mechanism of obvious importance to the host (56).

Diarrhea is an explosive response by the body, the purpose of which is to purge the GI tract of an unwanted, possibly harmful organism or substance. During such an event, the normal barriers to infection may be temporarily removed or inactivated, and other changes may occur in the unshielded epithelium, which directly or indirectly alter the host defenses.

EFFECTS OTHER THAN DIARRHEA CAUSED BY BACTERIA AND BACTERIAL TOXINS

Bacteria possess an array of pathogenic mechanisms which can lead to diarrhea. The complexity of the situation was recently reviewed (20). The processes can be placed in three basic categories: bacterial adherence (previously discussed), bacterial invasion and bacterial elaboration of enterotoxins.

Enterotoxins may be further classified into two groups according to functional criteria: classical enterotoxins (e.g., cholera toxin), which elicit fluid loss by known mechanisms with little or no morphologic alteration of the intestine, and cytotoxins, which elicit fluid loss by unknown mechanisms and severely alter intestinal morphology. Clinical studies often describe only the predominant organism isolated from the feces, failing to consider the possible involvement of secondary organisms. For instance, early reports state that V. cholera causes malabsorption phenomena (31), but Binder and Whiting (7) found that intestinal absorptive function remains intact during clinical and experimental cholera in which no mucosal morphologic changes are noted.

In contrast to the classical enterotoxins, the cytotoxins give a more defined picture. Studies on the effect of Klebsiella pneumoniae enterotoxin on intestinal transport in the rat were conducted by Klipstein et al. (30). Absorption of sugar, notably xylose, was inhibited during acute diarrhea caused by the heat-stable toxin. Using rabbit ileal loops, Binder and Whiting (7) showed that the enterotoxin of Shigella dysenteriae I impairs both sugar and amino acid transport. Likewise, the alpha- and delta-toxins (hemolysins) of Staphylococcus aureus inhibited amino acid transport in human Henle 407 intestinal cells in vitro, but the purified staphylococcal enterotoxins A and B did not (11). Salmonella typhimurium infections have also been shown to cause malabsorption of vitamin B_{12} (21) and to elicit a decrease in food and water intake (35), thus contributing to nutritional deficiency. The same observations have been made by clinicians on children with nonspecified diarrhea disease, in whom losses of energy (-160 Kcal/d) and protein (-3.0 g/d) due to decreased intake were observed. MacLean et al. (33) studied transient steatorrhea in infants with mild diarrhea and found that fecal fat excretion rose from 2.9 ± 1.4 g/d (before diarrhea) to 8.7 ± 3.1 g/d after diarrhea, resulting in a further loss of energy. Loss of endogenous amino acids during infantile diarrhea was studied by Ghadimi et al. (19). Compared with control and remission values, free amino acids were elevated 10-fold in stools of infants with diarrhea. Protein and total nitrogen losses were elevated during diarrhea, but to a lesser extent (4- and 2.5-fold, respectively). The loss of amino acids during diarrhea takes on special significance in light of Wannemacher's studies (58) on the role of various individual amino acids in the host response to infection. Energy metabolism is again involved; in an attempt to compensate for glucose loss, branched chain (leucine, isoleucine, valine) amino acids from the catabolism of skeletal muscle are used as an energy source or for synthesis of alanine or glutamine. Alanine may then be used by the liver as a substrate for gluconeogenesis. As will be discussed later, loss or deprivation of single essential amino acids may have deleterious effects on host defense mechanisms.

During diarrhea, malabsorption of nutrients occurs because of direct effects on the absorptive epithelium and because of the anorexia that often accompanies diarrhea. Nutrients lost because of malabsorption include sugars (monosaccharides), protein and amino acids, fat, vitamins, minerals and trace elements (reviewed in refs. 9 and 46). Diarrheas caused by bacteria, viruses, protozoan and other parasites all may cause malabsorption. The seemingly insignificant loss of even a single nutrient may have severe consequences on host defense mechanisms and may contribute to the vicious cycle of diarrhea-malnutrition-infection.

IMPACT OF DIARRHEA-INDUCED NUTRIENT LOSS ON HOST IMMUNITY

Hundreds of articles and numerous books have addressed the subject of nutrition and the immune response. Many clinical studies (e.g., on kwashiorkor and marasmus) were limited to the effects of severe malnutrition on the immune system. The effects of temporary loss or depletion of energy and...
certain key nutrients are difficult to measure owing to many variables such as methods used, inherent variation among individuals, etc. Nonetheless, such losses probably could adversely affect the rapidly developing immune system and other organ systems of the newborn genetically predisposed or the otherwise compromised individual such as the marginally malnourished.

In a recent review of immunodeficiency in undernutrition and overnutrition, Chandra (14) notes that the knowledge that immunity is compromised in nutritional deprivation states is relatively recent. Chandra (14) contrasts the immunological impact of severe malnutrition with moderate and marginal malnutrition, which could be induced at least temporarily by diarrheal episodes. Chandra further points out that within the marginally undernourished group, the immunological parameters that are monitored vary considerably from near normal to extremely low, again underscoring the diversity of individual nutrient deprivation. In affluent societies such as ours, obesity is the commonest type of malnutrition (14), and the rate of infection in obese persons is high. Subtle deficits in cell-mediated immunity and polymorphonuclear leukocyte function in obese individuals were reversed by the correction of iron and zinc deficiencies (14). Single nutrient deficiencies of iron, zinc, magnesium, selected amino acids and vitamins (A, E, folate, pyridoxine) demonstrated during diarrheal episodes all depress some aspects of the immune response (14).

More easily definable animal studies have verified observations of clinical conditions of selected nutrient loss and the immune response. Petro and Bhattacharjee (43) studied the effects of essential amino acid (leucine, isoleucine, valine, lysine) limitations on immune response parameters. In both male and female mice fed isoleucine- and valine-limited diets, the levels of complement component 3 (C3) were significantly lower than those in control mice. Loss or depletion of C3 suppresses IgA production in response to oral immunization (39). Serum IgM and IgA levels were higher in mice fed the experimental diets compared with mice fed control diets; this would be expected if endotoxin influx from indigenous gram-negative bacteria occurred from loss of slgA. The separate effects of malnutrition on serum IgA and slgA have also been shown in humans (51), i.e., serum IgA levels were elevated in children with protein-calorie malnutrition when slgA levels were at their lowest. Various dietary proteins are limited in one or more essential amino acids; soybean protein, for instance, is limited in leucine, threonine and valine, whereas wheat gluten is limited in lysine, isoleucine and threonine (22).

Petro and Bhattacharjee (44) carried their preliminary investigations one step further into a host-resistance system by studying the effects of selected amino acid depletion on resistance of mice to *S. typhimurium*. Limitation of any one of the essential amino acids isoleucine, valine and lysine was associated with increased mortality of both CF1 and Swiss-Webster mice from infection by two strains of *S. typhimurium* (44). Significant increases in proliferating *S. typhimurium* SR 11 recovered from livers and spleens 1 d after intraperitoneal injection of 300 bacteria correlated with mortality results, with leucine deprivation, in addition to isoleucine, valine or lysine deprivation also contributing to increased invasiveness. Isoleucine, valine or lysine limitation for 21 d also caused a decrease in splenocytes. In the mice, depressed serum C3 values were correlated with diets limited in valine, leucine or lysine, while depressed serum transferrin levels were correlated with limitation of valine or lysine (44). Serum transferrin levels correlate extremely well with the clinical grade of malnutrition (32). Some malnourished children with low serum transferrin levels died when an improved diet was administered. The low levels of transferrin were insufficient to bind the increased iron in the improved diet, resulting in increased circulating free iron, increased bacterial growth, septicemia and death (32).

The consequences of increased free iron caused by depressed transferrin levels and other conditions for both human and animal models have been reviewed by Weinberg (59). Basically, bacteria require iron for growth, but are limited in their use of iron because of the high association constant of transferrin for iron. Bacteria collect and absorb iron by means of siderophores, some of which can compete successfully with transferrin with regard to the iron association constant. Payne and Finkelstein (38) have suggested classifying the relative virulence of gram-negative organisms according to their response to iron. Recently, the virulence and high mortality associated with *V. vulnificus* infection have been correlated with production of siderophores by this bacterium, and with increased iron levels in infected persons (50).

Thus malabsorption and loss of essential nutrients caused by microorganism-induced diarrheal episodes cause transient depression of several immunologic mechanisms, which may then lead to greater susceptibility to more infection. Obviously, limitation of even a single essential nutrient can have a measurable impact. Several of the mechanisms most sensitive to nutrient depletion involve the GI barrier, the consequences of which will be discussed further.

**POSSIBLE HEALTH EFFECTS OF DIARRHEA-INDUCED LOSS OF INTACT GI BARRIER**

Microorganisms may alter intestinal morphology and function by means of toxic by-products (enterotoxins or cytotoxins), direct invasion of the epithelium by microorganisms, depression of immunologic functions caused by malabsorption or loss of nutrients during diarrhea or by the physical force of fluid loss during diarrhea.

Under normal circumstances, intact proteins may traverse the epithelium in small, carefully regulated amounts. This normal passage of large molecules maintains immunologic homeostasis by stimulating regulatory lymphocytes, which depress antibody formation in systemic humoral immunity and provide for immunologic memory to deal with larger insults. Also, intake of limited amounts of antigenic material stimulates cellular immune mechanisms to deal with organisms which successfully traverse the epithelium.

Gruskay and Cooke (24) studied the uptake of egg albumin in normal infants recovering from moderately severe diarrhea (unspecified cause). Five times more intact ovalbu-
rotizing enterocolitis, GI allergy, sudden infant death syndrome greatly to intact antigen uptake (55,57).

Numerous GI maladies, chronic intermittent diarrheas of un- and complete review of the potential contribution of syndrome, dermatitis, diarrhea and malabsorption. An ex-
clude inflammatory bowel disease, GI allergy, celiac possibly caused by increased macromolecule absorption in-
to loss of slgA production. Certainly, loss of slgA contrib-
utes to hidden antigens on mouse red blood cells caused by infection of the mouse with Pseudomonas aeruginosa. The mechanism involved polyclonal B cell activation in a manner identical to the lipopolysaccharides from gram-negative bacteria and other organisms listed by Garzelli et al. (18). Increases in autoantibody-producing cells in the absence of B cell proliferation indicated that polyclonal B cell activators do not act by simply increasing the number of autoantibody-producing cells, but by activating already programmed cells (normally suppressed) to differentiate into cells actively producing autoantibody (18).

The relevant question then becomes, Why don’t all persons with diarrheal disease or malnutrition develop chronic disease?

PREDISPOSING FACTORS TO CHRONIC DISEASE

Many persons suffer from sporadic diarrheal episodes and most do not develop long-term debilitating disease. But since many individuals do develop autoimmune disorders, vascular disease, heart disease, neoplasia and allergy, perhaps the role of diarrheal episodes in such persons has not been adequately assessed.

Undoubtedly, GI permeability is altered during diarrheal episodes and the uptake of macromolecules is increased. Likewise, diminution of slgA associated with diarrhea, owing to poor nutritional status before or malabsorption during and after the episode, also results in increased intake of antigenically active proteins. Bernstein and Ovary (6) discussed some of the factors affecting the uptake of proteins and resultant allergy. In their study, uptake was dependent on the molecular weight of a compound; all guinea pigs that received a hapten (m.w. 478) via gastric tube demonstrated a skin reaction at a site where sensitizing antibody to the hapten had been deposited 5 h earlier. Some guinea pigs fed ovalbumin (m.w. 44,000) responded with a skin reaction at a site where sensitizing antibody to ovalbumin had been de-
position, while others did not. In contrast, none of the animals in the group fed bovine gamma globulin (m.w. 175,000) reacted in the skin test system. The different reactions among individual animals fed ovalbumin may reflect such variables as intestinal transit time, degree of protein digestion, or the status of the GI tract at the time of oral challenge (6). Systemic allergic reactions to ingested antigens have been demonstrated clinically; in most instances, patients with reactions to food antigens had a history of atopic disease (23).
Infants who are allergic to cow’s milk often have a local reaction in the small intestine involving several immunologic mechanisms rather than a systemic response (49). The genetic predisposition of some persons to atopy is well documented.

In addition to molecular weight, other factors which may affect a protein’s allergenicity are ill defined. In allergy to fish, for example, of all the proteins extracted from cod muscle and blood, the bulk of the allergenicity is associated with single amino acid or multiple essential amino acids, a situation demonstrated to occur during diarrhea (7). In one study using mice, the regulatory anomalies induced by dietary manipulation involved altered ratios of helper-induced to suppressor-T lymphocytes (45). Protein-energy malnutrition in humans also induces a low T4/T8 (helper-inducer/suppressor cell) ratio (15). Altered ratios of this type are the principal immunologic abnormality in persons with acquired immune deficiency syndrome (47); these individuals also have an extremely high incidence of GI disorders from a variety of pathogens (41).

Circulating immune complex formation alone is insufficient to establish autoimmunity; another immunologic abnormality must exist as well, possibly caused by extraneous factors toxic to the immune system or dietary insufficiency. Another possibility is that the second factor involves genetic predisposition. Numerous autoimmune disorders such as rheumatoid arthritis, juvenile rheumatoid arthritis, diabetes, ulcerative colitis, multiple sclerosis, psoriasis and many others have been linked to the expression of certain human leukocyte antigens (HLA antigens). Persons with certain HLA antigens have a higher relative risk of developing autoimmune syndromes than those who express different antigens (reviewed in ref. 12). However, very few persons are aware of their HLA profile and are therefore unaware of their relative risk of acquiring autoimmune problems.

CONCLUSION

Not all factors and aspects of diarrheal disease, its link to nutritional factors and its contribution to chronic disease processes can be discussed in detail. The interrelationships involve areas of biological research which are rapidly evolving. Nonetheless, the evidence strongly suggests a causative relationship between diarrhea and certain long-term, chronic diseases. This area of research needs prospective research and novel approaches, and will require increased expenditures of research resources as well as a joint effort by researchers in nutrition, microbiology, pathology and immunology.

REFERENCES


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sociated lymphoid system in health and disease: a review. Pathology 10:3-16.
